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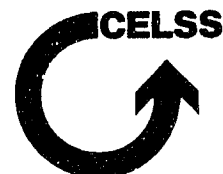
Plant Growth in Controlled Environments in Response
to Characteristics of Nutrient Solutions

C. David Raper, Jr.



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to Characteristics of Nutrient Solutions

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INTRODUCTION

The results of research during the second year of funding is summarized in this report. A brief summary of research accomplishments is followed in the appendices by more detailed descriptions of experimental results being prepared for publication in referred journals.

During the series of experiments completed in the first year, emphasis was given to environmental factors that alter the flux of carbohydrate from the shoot to the root system to support the absorption of nitrogen and the subsequent interaction between nitrogen uptake and plant growth. In these initial studies, only the NO_3^- form of nitrogen was utilized, and was supplied in adequate amounts to support maximum growth rates, to establish base line responses for evaluation during the second year of plant acclimation to stress conditions created by changes in solution temperature, nitrogen availability, and presence of NH_4^+ as a nitrogen source. In addition to these perturbations within the root-zone of plants growing in controlled environments, a dynamic mathematical simulation model of plant growth was validated.

Results of these studies have several implications for plant-culture systems in CELSS. (1) Over long periods of exposure, the ability of whole root systems to absorb NO_3^- is similar at cool and warm temperatures. Thus, although temperature of the root-zone of plants in a CELSS can be expected to remain relatively stable, the range of acceptable root-zone temperatures is wide. (2) If nitrogen supply in nutrient solutions is disrupted, an early response by vegetative plants is a decrease in rate of leaf initiation and expansion that is relatively more abrupt than the decrease in net CO_2 exchange rate per unit leaf area. Although the extent and speed of recovery in net CO_2 exchange rate upon resupply of nitrogen remains to be

determined, it is evident that the rapid reduction in leaf expansion and initiation upon interruption of nitrogen supply can have a prolonged effect on efficiency of lighted area. (3) In a CELSS system utilizing a flowing hydroponics nutrient supply, soybeans can tolerate NH_4^+ as a sole nitrogen source with no reduction in vegetative growth when pH of the solution is carefully controlled and environmental conditions remain stable. However, continuing experiments must be conducted to assess whether plants supplied with NH_4^+ can acclimate to a wide range of solution acidities or other environmental perturbations as readily as can plants supplied with NO_3^- . (4) The dynamic model being developed for plant growth is capable of predicting dry matter accumulation and partitioning in response to temporal changes in temperatures between 14 and 34 C with frequencies of change of less than one-half day.

SUMMARY OF RESULTS

Root Acclimation to Temperature (Appendix 1)

Biological membranes require a fluid physical state to function. The fluidity of biological membranes may be governed by metabolic regulation of lipid composition. In response to external stimuli, such as temperature, changes in metabolism and acyl-lipid saturation of membranes often are observed. In root tissues of several species, the ratio of polyunsaturated to saturated fatty acid concentration generally increases with decreases in temperature. At low temperature a decrease in membrane fluidity enhances polyunsaturated fatty acid formation, while at higher temperatures an increase in fluidity favors the synthesis of saturated acyl-units. Because of the apparent rapid response associated with changes in the ratio of polyunsaturated to saturated fatty acids in root membranes to changes in

temperature, changes in fatty acid composition have been proposed as an important factor in maintenance of biological activity in root systems, such as absorption of nutrient ions, at low temperature. However, the effect of temperature upon lipid composition could be only coincidental with tissue acclimation.

To evaluate the effects of temperature on fatty acid composition of soybean root systems, the root systems were divided into new roots developed during exposure to root-zone temperatures of 14, 18, and 22 C for 26 days and old roots developed prior to exposure to the temperature variable. New roots had a greater concentration of polyunsaturated fatty acids than old roots. The ratio of polyunsaturated to saturated fatty acid concentration in new roots exposed to 14 and 18 C peaked at 16 days and declined, while the corresponding ratio in old roots increased throughout the treatment period. Apparently, the response of fatty acid composition in old and new roots to low temperature was mediated by tissue aging or differentiation. These findings were contrary to the concept that modifications in fatty acid composition remain constant at lower temperatures.

The function of root tissues exposed to lower temperature was evaluated with respect to the ability of the root system to absorb NO_3^- . Over the relatively long periods of exposure, the ability of whole root systems to absorb NO_3^- was similar at cool and warm temperatures. The effect of cool temperatures on functioning of roots appeared to involve reductions in the rates of initiation and differentiation of young root tissues, or the morphological component of root functioning, rather than changes in membrane permeability related to alteration of fatty acid composition.

Root and Shoot Acclimation to Nitrogen Stress (Appendix 2)

Based on previous work, a scheme has been advanced to relate nitrogen uptake activity of roots to a balance between root and shoot functions. Based on considerations of the scheme for functional interdependence of roots and shoots, a primary effect of a reduction in uptake of nitrogen by roots and transport to shoots when external supply of nitrogen is interrupted would be an immediate decrease in both the rate of initiation and expansion of leaves and the net CO_2 exchange rate per unit leaf area. The effect on leaf initiation and expansion should be greater than the immediate effect on net CO_2 exchange rate. Because initiation and expansion of leaves are sinks, rather than sources, for photosynthate and are competitive with root sinks, a short term reaction to an interruption of nitrogen supply would be an enhanced availability of photosynthate in the shoot for transport to the roots. The enhanced transport of photosynthate to roots would continue until the net CO_2 exchange rates per plant begin to decline in response to eventual reductions both in net exchange rate per unit leaf area and total leaf area per plant. If these considerations are valid, a temporary period of absolute increase in root dry weight should immediately follow an interruption in nitrogen supply, but as the nitrogen stress continues, root growth rate eventually should decline. This decline in root growth rate, however, should be more gradual than the decreased rate of shoot growth so that shoot/root ratio is continually reduced over time.

To evaluate this scheme for root and shoot responses to interruptions in nitrogen supply, vegetative, non-nodulated soybean plants were grown for 21 days with 1.0 mM NO_3^- and then exposed to solutions containing 1.0, 0.1, or 0.0 mM NO_3^- for a 25-day treatment period. In nitrogen stressed plants, both CO_2 exchange rates per unit leaf area and expansion of total

photosynthetic leaf area per plant were decreased within a few days of treatment. The decrease in leaf expansion was a result of reduced emergence of new leaves and a decrease in expansion of leaves. As implicit from the conceptual scheme for root and shoot interdependence, not only was a greater portion of the dry matter production partitioned to roots with decreased availability of external nitrogen supply, but the growth rate of roots was enhanced by the nitrogen stresses for several days. These results, therefore, support the conceptual model for root and shoot functional balance as a mechanistic basis for modelling carbon and nitrogen flows in plants.

Growth Responses to NH_4^+ and NO_3^- Nutrition (Appendix 3)

The effect of NH_4^+ nutrition on the balanced interdependence of root and shoot functions in determining nitrogen uptake was examined in flowing solution culture. Vegetative soybean plants growing in controlled environment rooms were exposed during a 4-week treatment period to complete nutrient solutions in which nitrogen was supplied as either 1.0 mM NH_4^+ , 1.0 mM NO_3^- , or 0.5 mM NH_4^+ plus 0.5 mM NO_3^- . Acidity of nutrient solutions was constantly maintained at pH 5.8 by automated control. In separate experiments, radiation (PPFD of 700 and 325 $\text{mol m}^{-2} \text{s}^{-1}$) and CO_2 (400 and 1000 l l^{-1}) levels in the aerial environment were controlled to produce distinct steady-state rates of leaf and plant growth. Within each of the aerial environments, however, source of nitrogen did not alter either dry matter or nitrogen accumulation and partitioning within plants. The results support the conclusions that when solution acidity and other environmental conditions are controlled and stable during growth, NH_4^+ is an acceptable nitrogen source for soybeans and the presence of NH_4^+ does not in itself alter the balance between root and shoot in regulating uptake and

utilization of nitrogen during steady-state grown.

Preliminary Model Validation to Changing Temperatures (Appendix 4)

Temperature is among the most pervasive environmental factors affecting plant growth. If a model of the influence of temperature on plant growth is to be useful, the direct effects of temperature on essential physiological processes must be identified and precisely determined. In a dynamic simulation model being developed for plant growth, we have incorporated the effects of ambient temperature through the processes of photosynthesis, respiration, growth, and aging. We have assumed, however, that translocation processes for carbohydrates between source and sink are not directly dependent on temperature, but rather that translocation rates are responsive to temperature indirectly through the effects of temperature on the concentration of carbohydrate in the source pool and the size and metabolic activity of the receiving (sink) organ.

Although in previous experiments the dry matter accumulation and distribution predicted by the model corresponded closely to the measured values of plants grown under a wide range of constant temperatures in controlled environments, a temporal change of ambient temperatures can alter dry matter accumulation. Thus, before the model can be useful for CELSS applications, it must have a demonstrated capacity to predict behavior of plants under conditions of changing temperatures. To evaluate the capability of the model under changing temperatures, we measured growth of plants at 2 or 3-day intervals during a 5-week period when temperatures in controlled environments were changed at weekly and daily intervals and in ascending or descending sequences.

Simulations of dry matter accumulation and distribution in plant parts were carried out using the programmed changes in temperature for experimental

plant growth and compared with measured values. The behavior of the model generally was similar to the measured growth of plants for both descending and ascending sequences at weekly and daily frequencies of change. This indicates that, when other environmental conditions remain constant, the model is capable of reasonably predicting the total dry matter production under changing temperatures with a switching interval of less than one-half day. It also is evident from these results that the assumption of temperature independence of translocation processes is adequate for the purpose of modelling plant growth over the experimental range of temperatures of 14 to 34 C.

PUBLICATIONS

Papers in Referred Journals

- RUFTY, T. W., C. D. RAPER, JR., and W. A. JACKSON. 1981. Nitrogen assimilation, root growth, and whole plant responses of soybean to root temperature, and to carbon dioxide and light in the aerial environment. *New Phytologist* 88:607-619.
- RUFTY, T. W., W. A. JACKSON, and C. D. RAPER, JR. 1981. Nitrate reduction in roots as affected by the presence of potassium and flux of nitrate through the roots. *Plant Physiology* 68:605-609.
- RUFTY, T. W., R. J. VOLK, P. R. McCLURE, D. W. ISRAEL, and C. D. RAPER, JR. 1982. Relative content of NO_3^- and reduced N in xylem exudate as an indicator of root reduction of concurrently absorbed $^{15}\text{NO}_3^-$. *Plant Physiology* 69:166-170.
- RUFTY, T. W., C. D. RAPER, JR., and W. A. JACKSON. 1982. Nitrate uptake, root and shoot growth, and ion balance of soybean plants during acclimation to root-zone acidity.

Manuscripts Accepted for Publication

- RUFTY, T. W., W. A. JACKSON, and C. D. RAPER, JR. 1982. Inhibition of nitrate assimilation in the presence of ammonium. I. The moderating influence of potassium. *Journal of Experimental Botany* 33: in press.
- OSMOND, D. L., R. F. WILSON, and C. D. RAPER, JR. 1983. Fatty acid composition and nitrate uptake of soybean roots during acclimation to low temperature. *Plant Physiology* 71: in press.

Manuscripts in Review

- RUFTY, T. W., C. D. RAPER, JR., and S. C. HUBER. Assimilation and partitioning of carbon by soybean plants in response to nitrogen stress.
- RUFTY, T. W., C. D. RAPER, JR., and W. Á. JACKSON. Nitrogen assimilation of soybeans at different growth rates in response to ammonium and nitrate nutrition.
- WANN, M., and C. D. RAPER, JR. A dynamic model for plant growth: Response to changing temperatures.

APPENDIX 1

Fatty Acid Composition and Nitrate uptake of Soybean Roots
during Acclimation to Low Temperature

Deanna L. Osmond
Richard F. Wilson
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Biological membranes require a fluid physical state to function (23). The fluidity of biological membranes may be governed by metabolic regulation of lipid composition (23). In response to external stimuli, such as temperature, changes in metabolism and acyl-lipid saturation of membranes often are observed (2, 7, 12). In root tissues of several species (1, 5, 9, 11, 18, 21, 22), the ratio of polyunsaturated to saturated fatty acid concentration generally increases with decreases in temperature. At low temperature a decrease in membrane fluidity enhances polyunsaturated fatty acid formation, while at higher temperatures an increase in fluidity favors the synthesis of saturated acyl-units (10, 23).

Because of the apparent rapid response associated with changes in the ratio of polyunsaturated to saturated fatty acids in root membranes to changes in temperature (18), changes in membrane fatty acid composition have been proposed as an important factor in maintenance of biological activity in root systems at low temperature. Although this concept has been accepted widely, it recently has been questioned in view of the apparent similarity in whole root membrane fluidity and the transition temperature of membrane lipids demonstrated with several wheat cultivars grown at 2 and 22 C (17). Other reports (3, 6, 9, 16) also have suggested that changes in fatty acid composition of membranes are not required for cold acclimation of a number of plant species. Furthermore, NO_3^- uptake activity, measured over relatively long exposure periods for whole root systems of tobacco (14) and soybeans (19), was found to differ only slightly between warm and cool temperatures.

These findings by no means discounted the effect of temperature upon lipid composition of plant membranes. Lipid composition of biological tissues, however, could change not only in response to external stimuli, but also to internal processes (13). Root growth rate and morphological differentiation and aging of root tissues are internal processes responsive to temperature (24) and could have an impact upon lipid composition during acclimation of plants to temperature changes. Hence, the effect of temperature upon lipid composition could be only coincidental with tissue acclimation.

A distinction between the effects of temperature and morphological differentiation upon lipid composition has been difficult to ascertain. In one study, however, changes in fatty acid composition of whole root systems of soybeans in response to temperature occurred primarily in the youngest root tissues produced during the treatment period (11). The objective of this investigation was to determine the time-course of changes in fatty acid composition of old and new soybean root tissues exposed to different temperatures and the impact of those changes upon NO_3^- adsorption by the root systems.

MATERIALS AND METHODS

Pretreatment Conditions: Germinated soybean seedlings [*Glycine max* (L.) Merr. cv. Ransom] with radicle lengths between 8 to 12 cm were transplanted into three 210-liter continuous flow, hydroponic culture systems (15, 20) located in a phytotron growth room (8). During a 2-week pretreatment period, plants were grown at $26/22 \pm 0.25$ C day/night aerial temperatures and 9/15 h day/night durations. A PPFD of $700 \pm 50 \mu\text{mol m}^{-2}\text{s}^{-1}$ and PR of $11 \pm 1 \text{ W m}^{-2}$ between wavelengths of 700 to 850 nm were provided during the day period from a combination of fluorescent and incandescent lamps and were measured with a LI-COR LI-185 Quantum/Radiometer^{2/} and LI-1905 and LI-790 sensors at 1.15 m from the lamps. To repress floral expression, the night period included a 3-h interruption after 6 h by incandescent lamps with PR of $9 \pm 1 \text{ W m}^{-2}$ and PPFD of $70 \pm 7 \mu\text{mol m}^{-2}\text{s}^{-1}$. Atmospheric carbon dioxide, monitored by infrared gas analysis, was maintained at $400 \pm 25 \mu\text{l liter}^{-1}$. Relative humidity was approximately 70%. Temperature of the hydroponic system during pretreatment was controlled at 22 ± 0.5 C. Nitrogen was supplied as 1.0 mM NO_3^- in a complete nutrient solution (19, 20). Freshly prepared solutions in the culture system were used every two days. The pH of the solutions was automatically monitored and controlled at 5.8 ± 0.1 by additions of 0.1 N H_2SO_4 or 0.1 N Ca(OH)_2 .

Treatment Conditions and Sampling: At the end of the pretreatment period, temperatures of the culture systems were changed to 14, 18, and 22 ± 0.5 C for a 26-d treatment period. Other environmental conditions were the same as during the pretreatment period. Beginning at

initiation of treatment temperatures, two plants from each treatment were sampled at 2 to 3-d intervals. When the root systems sampled at treatment initiation were placed on a horizontal surface, primary and lateral roots were defined in a triangular pattern. A template was made from the dimensions of the triangle and used at subsequent samplings to distinguish and separate roots present upon initial exposure to temperature treatments (old roots) from roots initiated and developed during exposure (new roots). Root tissues and shoots were frozen immediately, freeze-dried, weighed, and ground for analysis.

Analytical Procedures: Total nitrogen in all plant tissues was assayed by a modified Kjeldahl procedure that digests all nitrogenous compounds, including NO_3^- , to NH_4^+ (13) for colorimetric determination (4). Root lipid composition of 1 g samples of freeze-dried tissue was determined as previously described (25). In a preliminary study, lipids in extracts of fresh and freeze-dried subsamples of root tissues were compared. The lipid composition of freeze-dried tissue was highly representative of composition of fresh tissue.

Growth Analysis: Natural logarithms of dry weights of roots and nitrogen contents of whole plants were fitted to linear regression equations as a function of days after initiation of treatments. The slope of these regressions express the relative rate of root growth and NO_3^- uptake during treatment. Specific NO_3^- uptake rates were calculated for each sampling date and expressed as meq of NO_3^- absorbed per g root dry weight per d (19, 20).

RESULTS AND DISCUSSION

Dry matter accumulation in the total root systems of soybeans increased exponentially (Fig. 1) with root RGR during the treatment period of 0.118, 0.116, and 0.123 g g⁻¹d⁻¹ at 14, 18, and 22 C, respectively. Differences in dry matter accumulation among treatments were significant at each date from 19 to 26 DAT (days at temperature). Although dry matter did continue to accumulate with thickening of the old roots present prior to exposure to the temperature differential, most of the difference in total root dry weight among temperatures was attributable to changes in growth of new roots (Fig. 1, inset). The specific uptake rates of NO₃⁻ during the treatment period were 1.31, 1.26, and 1.31 meq NO₃⁻ (g root dry weight)⁻¹d⁻¹ at 14, 18, and 22 C, respectively. Thus, the uptake activity per unit root mass was not affected by temperature and the effect of root-zone temperature on uptake of nitrogen could be related to a change in the amount of root tissue.

In accord with the changes in root dry weight, total acyl-glycerolipid content present in both old and new root tissues also increased during the treatment period (Table I). Within a temperature treatment no significant differences occurred in net lipid accumulation between old and new root tissues at respective dates from 7 to 19 DAT. New root tissues, however, contained a greater mass of lipid than old tissues at 21 to 26 DAT. Although the net lipid accumulation at 26 DAT in new or old roots was significantly greater with increased temperature, new root tissues contained 1.6 ± 0.03 times more lipid than old tissues in each temperature treatment.

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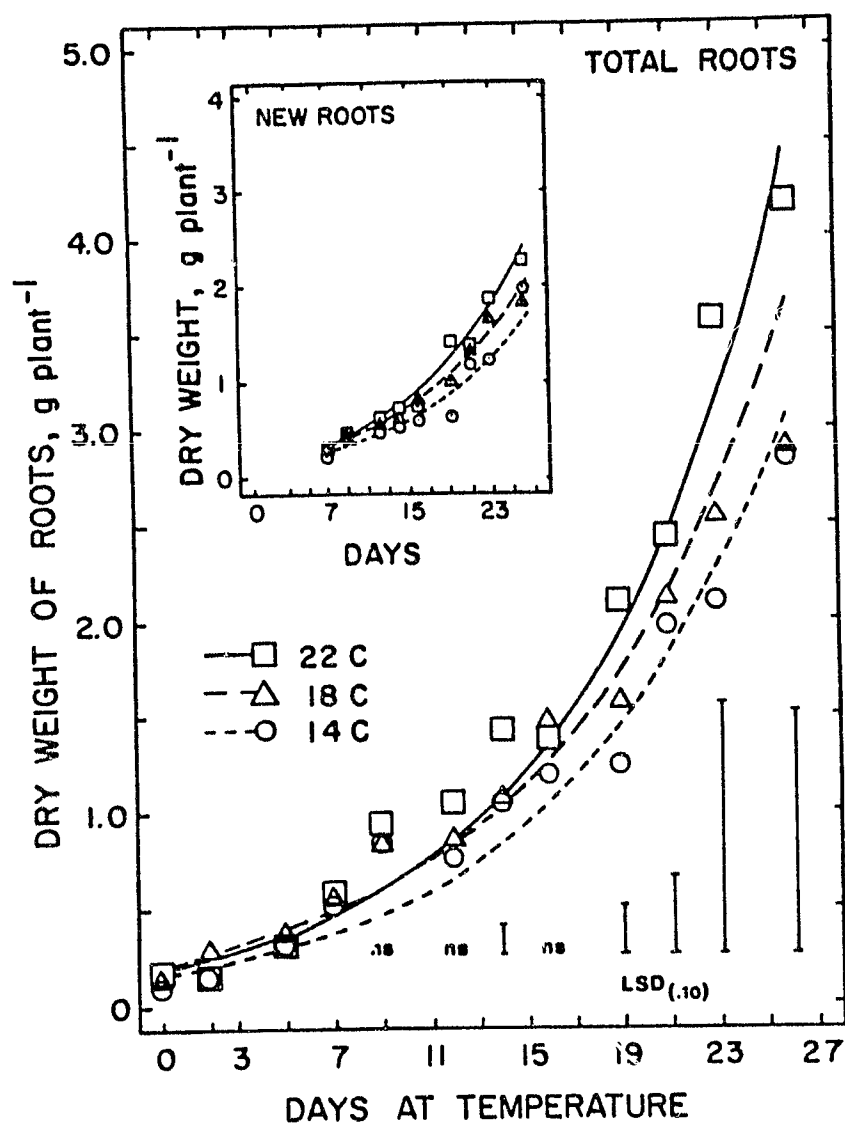


Fig. 1. Dry weights of total root systems at root-zone temperatures of 14, 18, and 22 C. Dry weights of new roots are shown in insert.

Table I. Accumulation of Lipid in Soybean Roots Exposed to Different Temperatures

Root Type ^a	DAT ^b	Temperature Treatment		
		14 C	18 C	22 C
	d	mg lipid ^c		
Old	7	40.2	43.0	40.2
	12	63.7	58.2	45.7
	16	79.0	80.0	84.6
	21	105.2	120.5	106.5
	26	164.8	174.4	197.8
New	7	37.4	58.1	62.3
	12	69.3	77.5	90.0
	16	103.9	119.1	114.9
	21	122.0	188.4	183.0
	26	234.2	278.6	316.0
	LSD 0.025 ^d	35.6	46.8	53.2

a: Old root tissues present at the initiation of treatment and root tissues initiated during the treatment period.

b: DAT, days at temperature

c: Summation of values for old and new roots respectively at a given date represent the amount of lipid extracted per root. Values at 9, 14, 19, and 23 DAT are not reported to simplify tabular presentation but were included in calculation of LSD.

d: LSD between and among root types within a temperature. LSD_{0.025} between and among temperatures within a root type is 25.4 for old roots and 41.6 for new roots.

Further characterization of root lipids revealed distinct differences in acyl composition between old and new roots. The predominant fatty acids present in root tissues were palmitic (16:0), linoleic (18:2), and linolenic (18:3) acid. The summed concentration (mole %) of 16:0, 18:2, and 18:3 was significantly greater in new than in old roots at each date within a temperature treatment except at 21 to 26 DAT at 22 C (Table II). The summed concentration of 16:0, 18:2, and 18:3 in the respective tissues also increased by significant levels between 7 to 26 DAT within each treatment. Differences among temperatures, however, were observed within the separate fatty acids.

The degree of acyl-lipid unsaturation of root tissues was expressed by calculation of a double bond index (DBI). The DBI values of lipids from old and new roots increased with time at all temperatures (Table III). In old roots, DBI values were not different among temperatures at 26 DAT; however the initial rates of increase in DBI values tended to increase as temperature decreased. For new roots, DBI values at 26 DAT were significantly greater as temperature decreased. In further contrast to older tissues, the observed DBI values in new roots peaked at 16 DAT for 14 and 18 C but did not change significantly with time at 22 C. Values of DBI for old and new roots at 26 DAT were not significantly different among temperatures.

The relative response of 18:2 and 18:3 concentration to temperature is expressed in Table IV by the mole % (18:2/18:3). At 7 DAT 18:2/18:3 ratios were significantly greater in old roots than in new roots, but not different among temperatures. Between 7 and 26 DAT the ratios declined by significant levels in all tissues except new roots exposed to 22 C. At 26 DAT there was no difference in the 18:2/18:3 ratio

Table II. Combined Concentration of Predominant Fatty Acids in Soybean
Roots Exposed to Different Temperatures

Root Type	DAT	Temperature Treatment		
		14 C	18 C	22 C
	d	mole % (16:0 + 18:2 + 18:3) ^a		
Old	7	81.8	81.3	81.2
	12	81.8	82.3	83.8
	16	82.9	86.8	86.3
	21	83.6	87.8	89.6
	26	84.5	89.2	91.0
New	7	88.4	88.7	89.1
	12	89.4	90.8	90.7
	16	90.2	92.1	91.6
	21	92.7	92.3	91.8
	26	93.1	92.4	92.1
	LSD 0.025 ^b	3.0	2.7	2.5

a: Values at 9, 14, 19, and 23 DAT are not reported to simplify
tabular presentation but were included in calculation of LSD.

b: LSD between and among root types within a temperature. LSD_{0.025}
between and among temperatures within a root type is 1.8 for
old roots and 0.8 for new roots.

Table III. Relative Degree of Unsaturation of Lipids in Soybean Roots
Exposed to Different Temperatures

Root Type	DAT	Treatment Temperature		
		14 C	18 C	22 C
	d	Double bond index		
Old	7	98.0	94.7	94.9
	12	120.5	110.0	98.4
	16	129.0	127.5	109.1
	21	141.3	136.2	119.9
	26	153.5	152.5	150.5
New	7	151.6	150.0	153.5
	12	171.3	162.8	157.0
	16	184.4	177.5	156.6
	21	179.0	173.7	161.9
	26	174.4	168.7	159.4
	LSD 0.025 ^b	18.5	18.8	18.9

a: Double bond index is calculated as mole % $[(18:1)(1) + (18:2)(2) + (18:3)(3)]$. Values at 9, 14, 19, and 23 DAT are not reported to simplify tabular presentation but were included in calculation of LSD.

b: LSD between and among root types within a temperature. $LSD_{0.025}$ between and among temperatures with a root type is 11.1 for old roots and 5.7 for new roots.

Table IV. Relative Relation Between Polyunsaturated Fatty Acids in
Soybean Roots Exposed to Different Temperatures

Root Type	DAT	Temperature Treatment		
		14 C	18 C	22 C
	d	mole % (18:2/18:3) ^a		
Old	7	1.7	1.7	1.7
	12	1.1	1.6	1.7
	16	0.9	1.2	1.6
	21	0.9	1.1	1.6
	26	0.8	1.0	1.4
New	7	1.4	1.4	1.4
	12	1.4	1.2	1.3
	16	1.0	1.1	1.3
	21	0.8	1.0	1.3
	26	0.8	1.0	1.3
	LSD 0.025 ^b	0.20	0.18	0.12

a: Values at 9, 14, 19, and 23 DAT are not reported to simplify
tabular presentation but were included in calculation of LSD.

b: LSD between and among root types within a temperature. LSD_{0.025}
between and among temperatures within a root type is 0.2 for
old roots and 0.1 for new roots.

between old and new roots within a temperature; however, the ratios declined as temperature decreased. Hence, these data indicated that net levels of 18:3 relative to 18:2 increased as temperature decreased.

Use of the R_1 ratio, mole % $(18:2 + 18:3)/(16:0)$, emphasized the balance between saturated and polyunsaturated fatty acids in old and new root tissues (Figs. 2, 3, 4). The trends shown by these data accentuated the patterns indicated by DBI calculations. In old roots, R_1 values gradually increased during the treatment period at all three temperatures. At 7 DAT R_1 values from old tissues were not different between treatments ($\bar{X} = 0.86 \pm 0.02$). At 26 DAT the respective R_1 values had increased by significant margins within each treatment, although there were no differences among temperatures ($\bar{X} = 2.07 \pm 0.07$). In new roots at 7 DAT R_1 values were greater as temperature decreased, but were not different among temperatures at 26 DAT ($\bar{X} = 2.48 \pm 0.06$). In addition R_1 values peaked at 16 DAT in new roots exposed to 14 C ($R_1 = 3.52$) and 18 C ($R_1 = 3.20$), whereas there were no significant differences between any of the R_1 values (7 to 26 DAT) in new roots at 22 C. Hence, at 14 and 18 C the R_1 values for new roots indicated that concentration of polyunsaturated fatty acids relative to the major saturated fatty acid increased significantly between 7 and 16 DAT and then decreased significantly from 16 to 26 DAT.

In an attempt to distinguish temperature and tissue aging effects upon root fatty acid composition, R_1 values expressed on a dry weight basis were compared among temperatures (Fig. 5). As shown, the response to temperature was quite apparent but was not sustained as the respective tissues increased in age. At 7 DAT there were no significant

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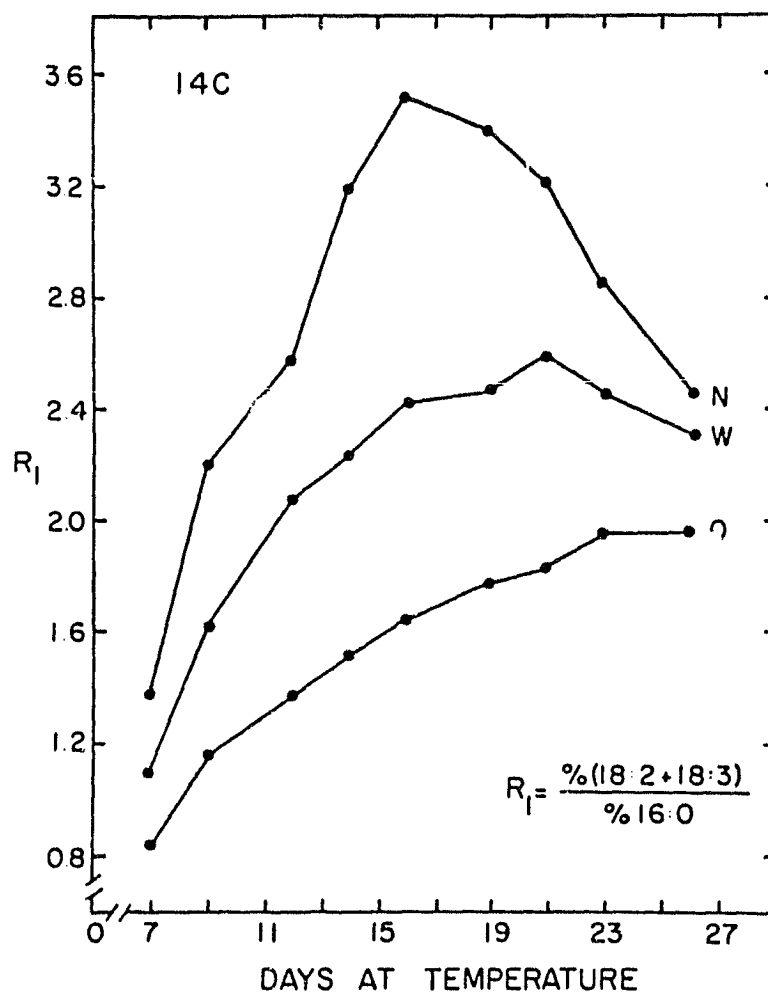


Fig. 2. Changes in R_1 ratio in old (O), new (N), and whole (W) root tissues during treatment at 14 C.

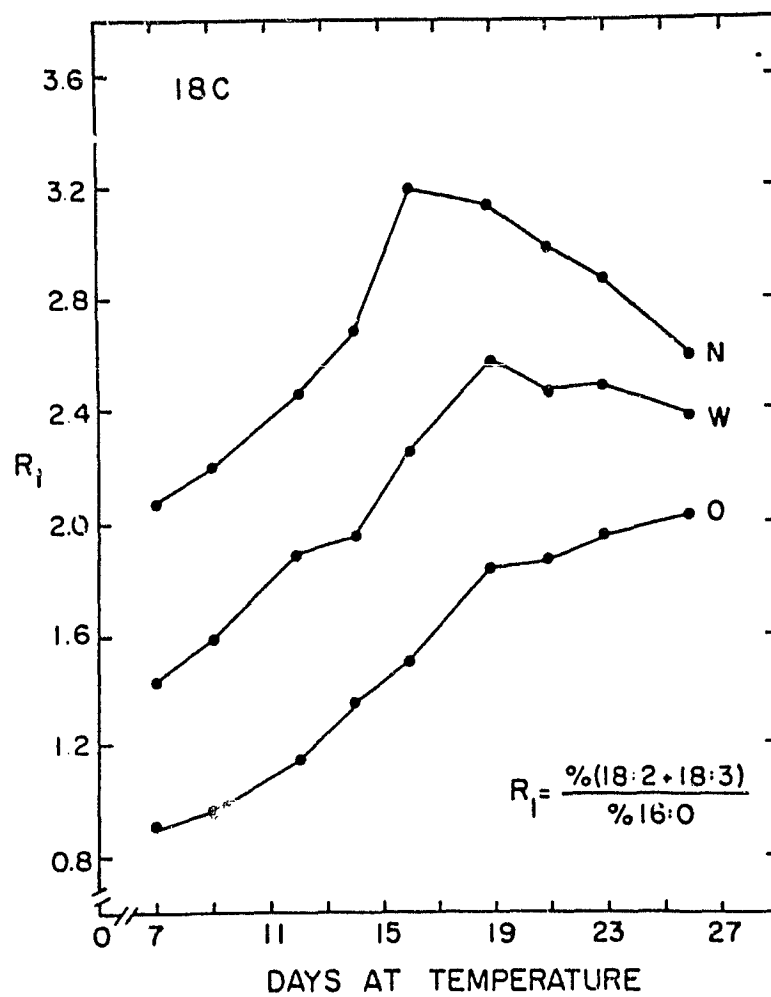


Fig. 3. Changes in R_1 ratio in old (O), new (N), and whole (W) root tissues during treatment at 18 C.

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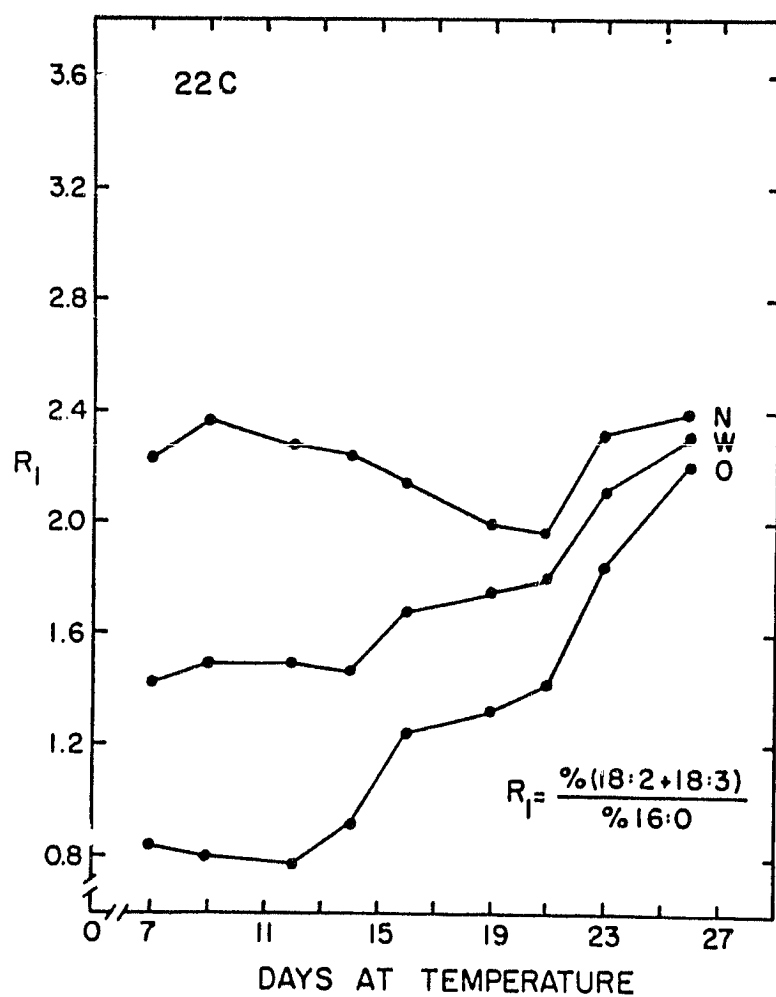


Fig. 4. Changes in R_1 ratio in old (O), new (N), and whole (W) root tissues during treatment at 22 C.

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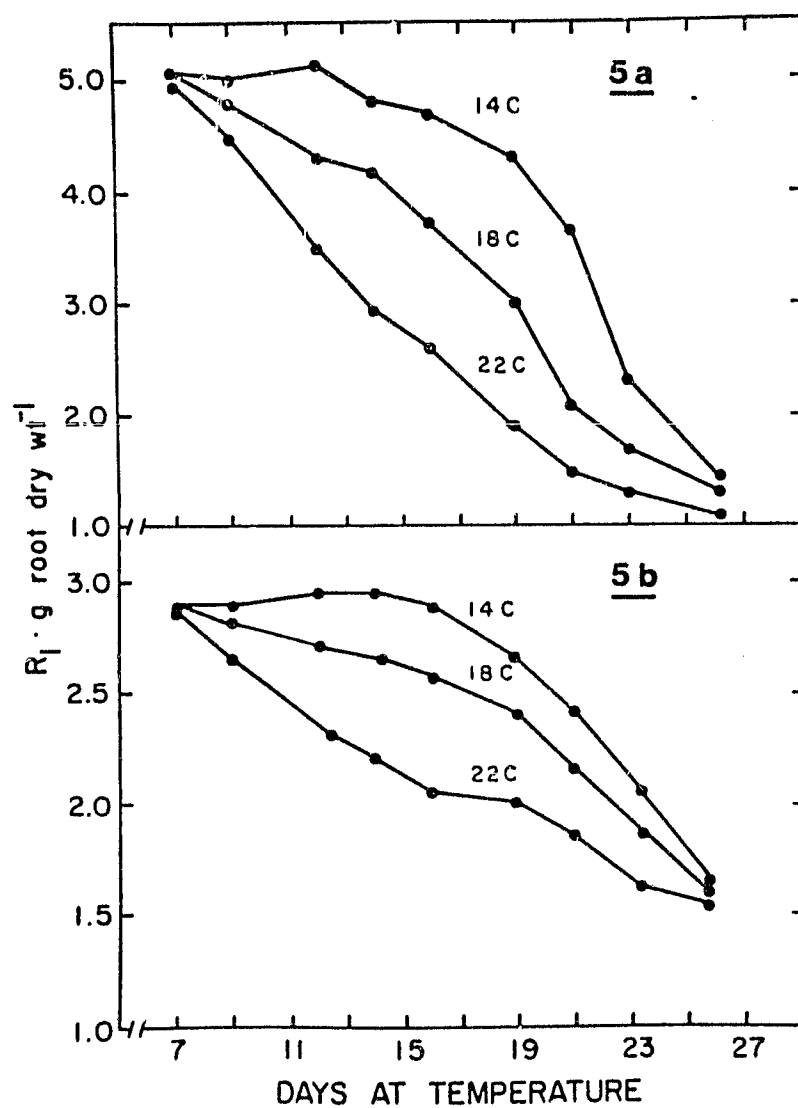


Fig. 5. Comparison of R_1 ratios expressed on a dry weight basis between new roots (5a) and old roots (5b) during treatment at 14, 18, or 22 C.

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differences in R_f values among temperatures for old or new roots. The greatest differences among temperatures for old and new roots occurred at 16 DAT. At 26 DAT there were no significant differences between any of the values at any temperature. Hence, the response of soybean root fatty acid composition to temperature diminished as tissues increased in age.

While a primary response of developing root tissues to cool temperatures may be an alteration of membrane fluidity associated with temperature-dependent changes in endogenous fatty acid composition, based on these results such changes appear to be relatively rapid, to have short term duration, and to be a function of tissue age. Indeed, had the treatment periods of this experiment been extended, it is possible that the relative concentrations of major saturated and polyunsaturated fatty acids of new and old root tissues would become indistinguishable, regardless of temperature. The results of this report concur with conclusions of others (3, 6, 9, 16, 17) that changes in membrane fatty acid composition are not a prerequisite for acclimation to cool temperature. In addition, these results indicate that trends in fatty acid composition of whole root systems induced by a change in temperature do not plateau at a steady-state level until growth temperature again is changed. Changes in fatty acid composition in response to temperature were mediated by maturation of root tissues. Cool temperatures reduced both the rate of initiation of young root tissues and also the rate of differentiation of new tissue. Consequently, over relatively long periods of exposure to a temperature,

acyl-lipid composition of whole root systems should be similar at cool and warm temperatures. Certainly, the specific uptake rate of NO_3^- as an indicator of activity of the whole root system was not different at root-zone temperatures of 14, 18, and 22 C over a 26-d exposure period.

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FOOTNOTES

1. Cooperative investigations of the North Carolina Agricultural Research Service and the USDA-ARS, Raleigh, NC. Paper No. 8100 of the Journal Series of North Carolina Agricultural Research Service, Raleigh, North Carolina 27650. The research was supported in part by Grant NCC 2-101 from NASA.
2. Mention of a trademark or a proprietary product does not constitute a guarantee or warranty of the product by the North Carolina Agricultural Research Service or by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that might also be suitable.
3. Abbreviations: 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; DAT, days at temperature; DBI, double bond index; GC, gas chromatography; PPFD, photosynthetic photon flux density; PR, photomorphogenic radiation; R_1 , mole % $[(18:2 + 18:3)/16:0]$; RGR, relative growth rate; RAR, relative accumulation rate.

APPENDIX 2

Assimilation and Internal Partitioning of Carbon by Soybean Plants
in Response to Nitrogen Stress

Thomas W. Rufty, Jr.

C. David Raper, Jr.

Stephen C. Huber

INTRODUCTION

The rate of assimilation of nitrogen is an important determinant of plant growth and development, and a primary means by which nitrogen influences growth is alteration of plant acquisition and internal distribution of carbon. Early investigations revealed that the predominant effect of suboptimal rates of nitrogen assimilation was a marked decrease in leaf area expansion which limited development of photosynthetic capacity (e.g. Gregory, 1926; Crowther, 1934; Watson, 1947; Morton and Watson, 1948). More recent experiments have clearly shown that nitrogen deficiency also can result in decreased CO₂ fixation rates per unit of leaf area (see Nair, 1972 and 1975 for reviews).

Alterations in the rate of nitrogen assimilation also apparently can result in modification of partitioning of carbon within the plant, as implied by differential effects on growth of plant parts. A common observation is that in plants exposed to suboptimal levels of nitrogen, shoot growth is inhibited to a greater extent than root growth and in some instances an initial stimulation of root growth has even been observed (Brouwer, 1962; Ryle, 1970; Clement, Jones, and Hopper, 1978; Drew, Sisworo, and Saker, 1979). Decreased shoot to root ratios with decreasing supply of external nitrogen have been noted in long term experiments (Raper et al., 1977; Ingestad and Lund, 1979; Sionit et al., 1981). Therefore, adjustments in partitioning of carbon between the shoot and root apparently can be maintained during extended growth periods and could be characteristic of a given nitrogen supply.

The effect of limited nitrogen availability on photosynthetic carbon assimilation and endogenous partitioning of carbon has rarely been characterized in the same experiment. As a consequence, an integrated view of the interrelationship among nitrogen and carbon processes and their involvement in alteration of growth and development of the whole plant has not emerged. Accordingly, in the investigation reported herein, the changes in plant acquisition and internal partitioning of carbon resulting from an imposed stress are evaluated collectively over an extended growth period. General cause and effect relationships are advanced to explain how nitrogen-carbon interactions and other, associated factors could regulate the whole plant growth response.

MATERIALS AND METHODS

Soybean seeds [Glycine max (L.) Merrill, 'Ransom'] were germinated in paper towels at 25°C and 98% relative humidity in a controlled environment chamber in the North Carolina State University phytotron unit of the Southeastern Plant Environment Laboratory (Downs and Bonaminio, 1976). The seeds were kept moist by capillary action from a 0.1 mM CaSO_4 solution. After 3 d, 24 seedlings, selected for radicle lengths between 8 and 12 cm, were placed into each of three 210 liter continuous-flow, hydroponic culture systems with temperature (Osmond, York, and Raper, 1981) and pH control (Ruffy, Raper and Jackson, 1982). The culture system was located in a growth room programmed for 26/22°C day/night temperature, a 9 h day period, and a 15 h night period. Photosynthetic photon flux density (PPFD) of $700 \pm 50 \mu\text{E m}^{-2}\text{s}^{-1}$ between wavelengths of 400 to 700 nm, with a photomorphogenic radiation (PR) of 12 W m^{-2} between wavelengths of 700 to 850 nm, was provided during the 9 h day period from a combination of fluorescent and incandescent lamps at an input wattage ratio of 10:3. The night period included a 3 h interruption by incandescent lamps to effect a long-day photoperiod and repress floral expression. The PPFD during the 3 h interruption of the night period was $70 \pm 10 \mu\text{E m}^{-2}\text{s}^{-1}$ with 10 W m^{-2} of PR between wavelengths of 700 to 850 nm. The ambient CO_2 concentration was maintained at $400 \pm 25 \mu\text{l/l}$. The light and CO_2 conditions used are sufficient to sustain whole plant growth rates which exceed those of field and greenhouse soybean plants.

During a 3 week pretreatment period, culture soln temperature was maintained at $24 \pm 0.5^{\circ}\text{C}$. The soln pH was maintained at 5.8 ± 0.1 by additions of 0.01 N $\text{Ca}(\text{OH})_2$ or 0.01 N H_2SO_4 . Nutrient concn in solution were 1.0 mM NO_3^- , 0.25 mM H_2PO_4^- , 1.25 mM K^+ , 1.0 mM Ca^{2+} , 1.0 mM Mg^{2+} , 2.0 mM SO_4^{2-} , 17.0 μM B, 3.0 μM Mn, 0.3 μM Zn, 0.1 μM Cu, 2.2 μM Cl, 0.04 μM Mo, and 1 mg Fe/liter as Fe-EDTA. The soln were changed every 2 d to avoid depletion effects.

Treatments were started 3 weeks after germination, when the sixth trifoliolate leaf was unfolding and plants were into the exponential growth phase. Plants in each culture system were exposed to treatment soln containing 1.0, 0.1, or 0.0 mM NO_3^- for a 25 d experimental period. All other nutritional and environmental conditions were the same as in the pretreatment. Again soln were changed every 2 d to avoid depletion effects. In addition, soln NO_3^- conc were monitored daily and supplemented when necessary.

During the experimental period, 3 or 4 plants were sampled from each nitrogen treatment at intervals specified in the results section. At each sampling, leaf number was determined as number of trifoliolate plus unifoliolate leaves, and leaf area measured photometrically with a LI-COR* LI-300 Area Meter. Plants were separated into leaves, stems (including petioles) and roots. The plant parts were frozen immediately, freeze-dried, and ground.

Total nitrogen was assayed by a modified Kjeldahl procedure to digest all nitrogenous compounds, including NO_3^- , to NH_4^+ (Nelson and Somers, 1973). The NH_4^+ was then determined colorimetrically

* The use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Research Service of the products named nor criticism of similar ones not mentioned.

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(Cataldo, Schrader, and Youngs, 1974). After hot water extraction, NO_3^- nitrogen was analyzed by a non-automated modification of the method of Lowe and Hamilton (1967). Reduced nitrogen was calculated as the difference between total and NO_3^- nitrogen.

Net CO_2 exchange rate (CER) of leaves from the upper and lower portions of the main stem from plants in each nitrogen treatment were measured throughout the experiment. CER was measured with a differential infrared CO_2 analyzer equipped with a clamp-on Plexiglas cuvette enclosing the upper and lower surfaces of a 10 cm^2 area of an attached leaf. Air at the same temperature and CO_2 concentration as ambient air of the growth chamber was passed through the cuvette. The cuvette remained in place for one minute while differences between CO_2 concn in incoming and exhaust air streams were monitored. When CER of shaded, lower-stem leaves was measured, the leaf canopy was pushed aside and shading effects minimized.

RESULTS

Nitrogen Stress

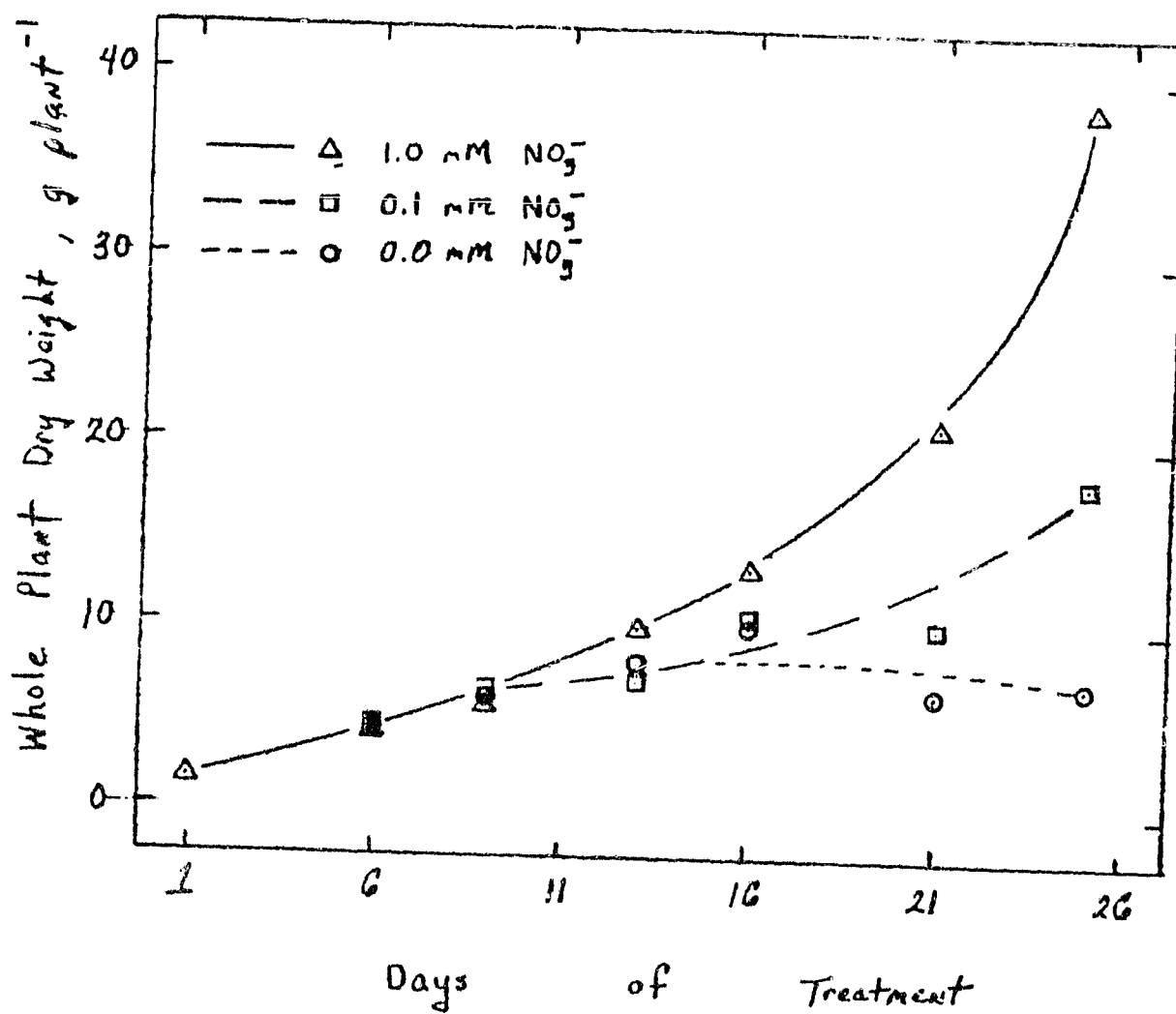
Vegetative soybean plants in the exponential growth phase were exposed to 1.0, 0.1, or 0.0 mM NO_3^- in otherwise complete nutrient solutions. It was suggested by Greenwood (1976), that the severity of nitrogen stress can be assessed as the effect on whole plant growth. Using this criterion, the 0.1 and 0.0 mM NO_3^- treatments effectively induced different degrees of nitrogen stress (Fig. 1). Whole plant growth in all treatments was similar for about 9 d after the start of treatments; however, after that time, growth of plants exposed to 0.1 mM NO_3^- proceeded at a rate which was considerably less than that of control plants, and little further growth was noted in plants exposed to the treatment solution without NO_3^- .

The Canopy Response

An early response to nitrogen stress was a decline in CO_2 exchange rates per unit of leaf area (CERs) throughout the canopy. Figure 2 shows the decreases in CER which occurred in older and younger fully expanded leaves and which were more severe in the absence of an exogenous supply of NO_3^- . Even though decreases in CER were noticeable within 7 d, decreases in whole plant d.wt were not apparent until 13 d after the start of treatments (Fig 1), which likely reflects the difference in sensitivity of the measurements. Differences in increments of dry matter accumulation initially are small relative to the total amount of dry matter already present, and therefore, are easily 'masked' by variability in total dry matter from plant to plant.

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Fig. 1



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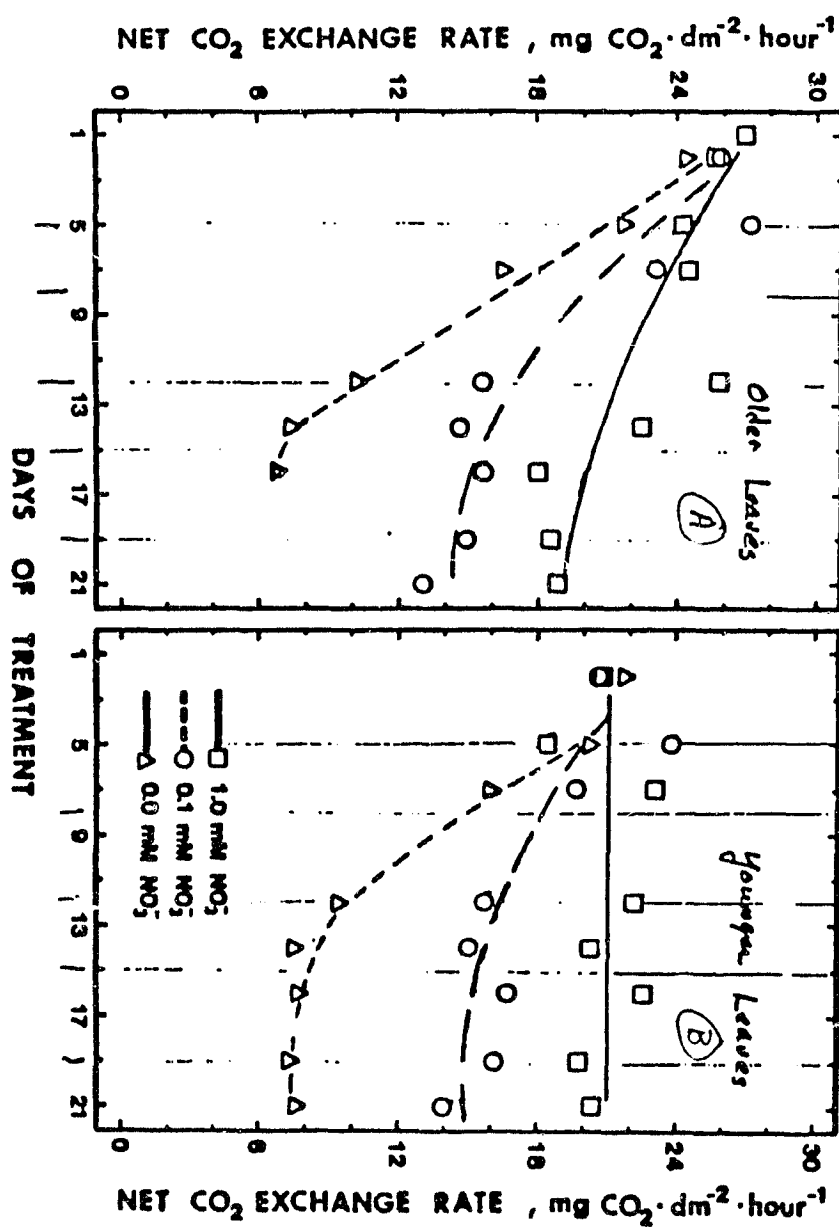


Fig. 2

Imposition of nitrogen stress also resulted in a restriction of emergence of new leaves and expansion of individual leaves. At 0.1 mM NO_3^- , the number of leaves per plant increased throughout the treatment period, but at a markedly slower rate than in control plants (Fig 3A). In the absence of solution NO_3^- , leaf emergence was severely restricted throughout. In both nitrogen stress treatments, the restriction was noticeable by 6 d after the start of treatments. Compared to leaves of control plants, leaves of nitrogen stressed plants expanded at a decreased rate and were considerably smaller at full expansion, as shown in Figure 3B, which is a plot of leaf area of the fifth and sixth trifoliolates on the main stem, combined. The effect on the expansion rate was apparent after 6 days. In addition to decreased leaf emergence and expansion, there was a distinct decrease in secondary stem development with increased nitrogen stress (data not shown). Collectively, these growth inhibitions resulted in a marked decrease in total leaf area per plant (Fig 3C).

Dry Weight Distribution Within the Plant

Partitioning of d.wt among plant parts was effected differentially by nitrogen stress. The d.wt accumulation patterns of leaves, stems, and roots of control plants are illustrated in figure 4A. Relative to accumulation of d.wt in the unstressed, control plants, accumulation in leaves in both nitrogen stress treatments was noticeably restricted by 6 d after the start of treatments (Figs 4B and C). Accumulation of d.wt in stems also was adversely effected, but the restriction was delayed somewhat. In contrast to the restriction of growth in the shoot, d.wt accumulation in roots was initially enhanced in both

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Fig. 3a

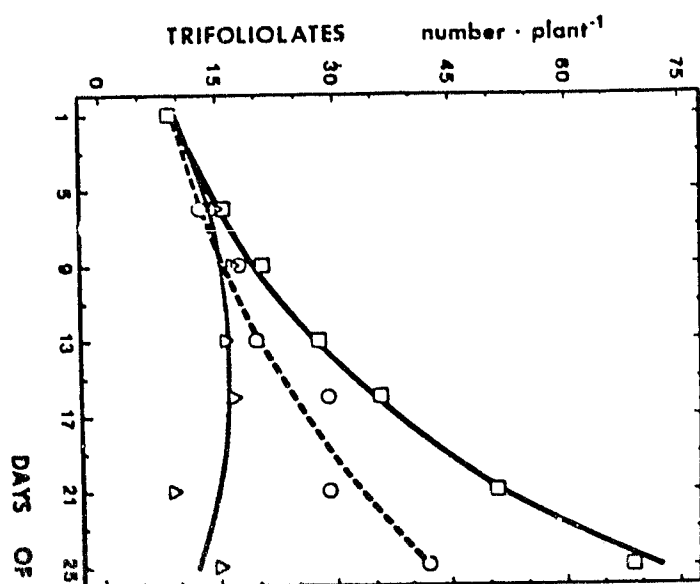
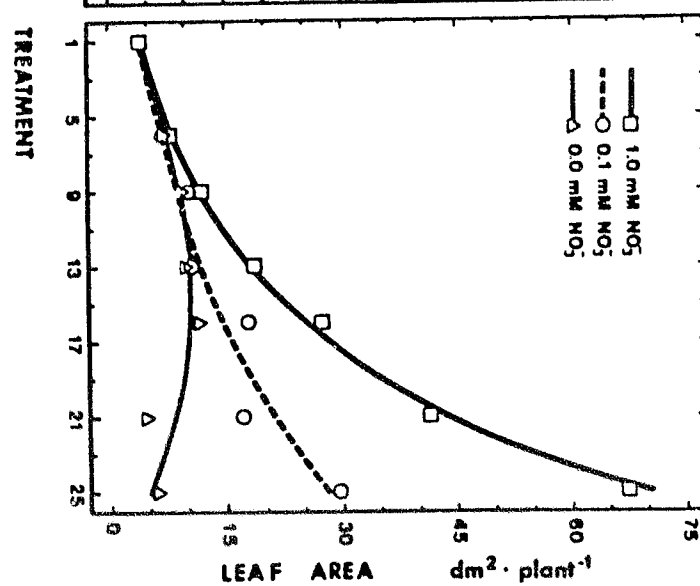
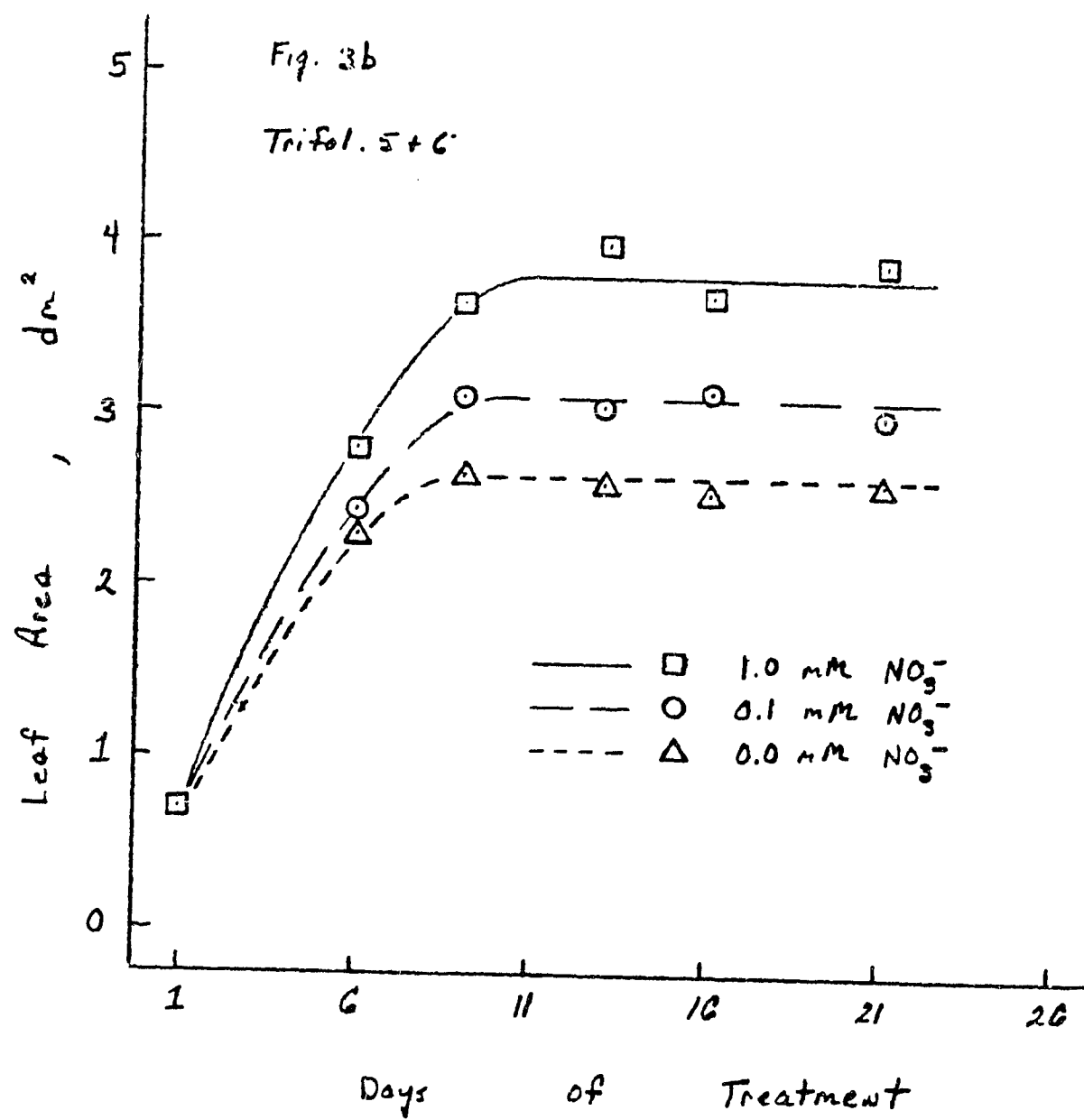
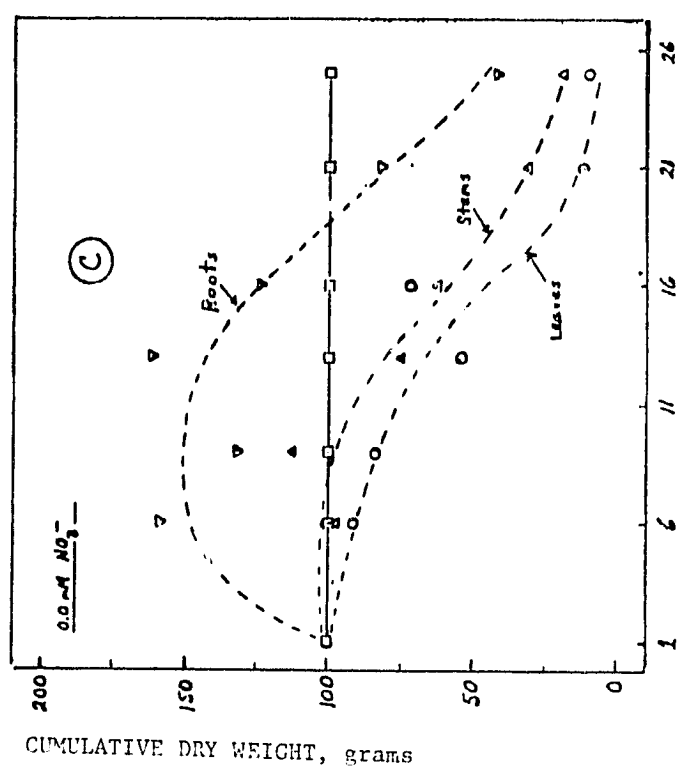
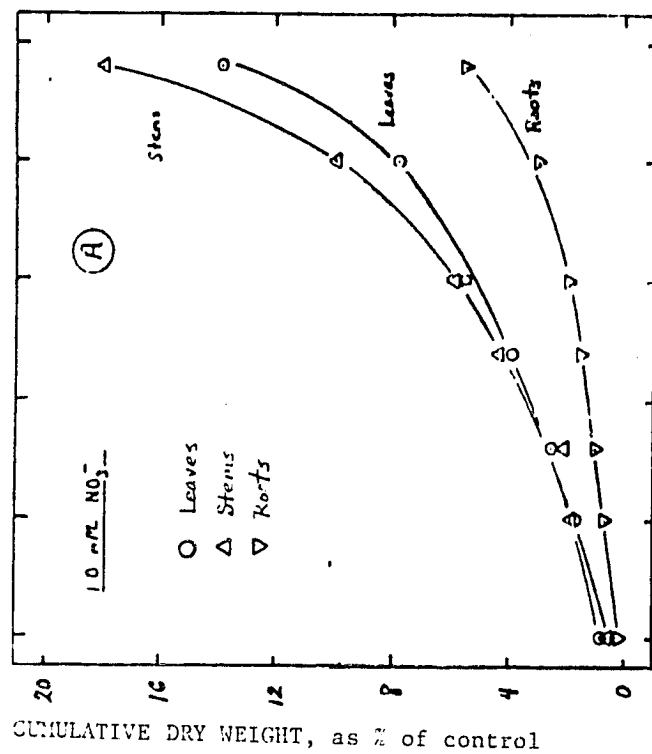
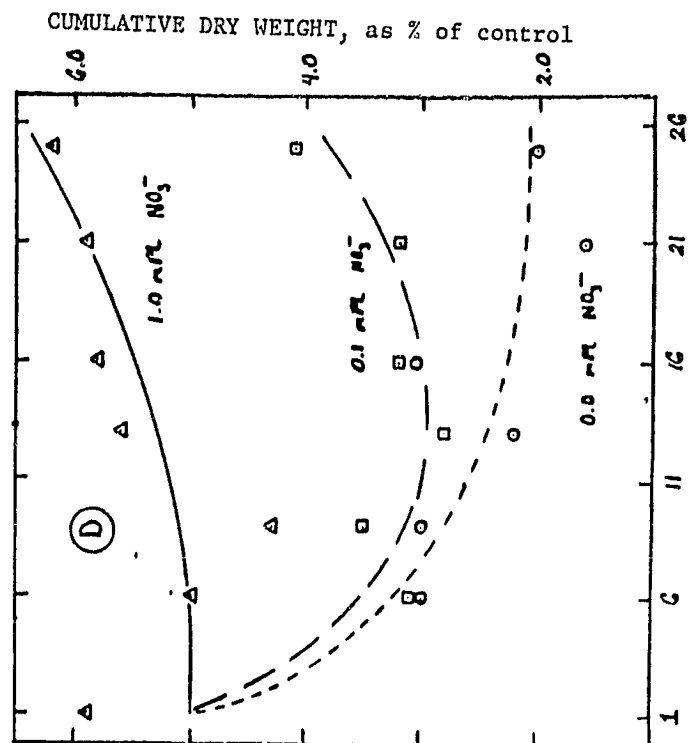
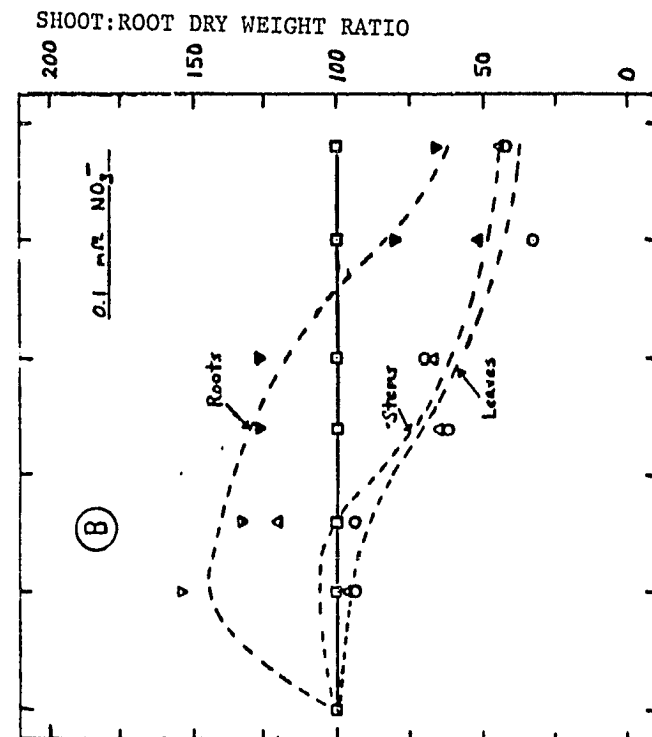


Fig. 3c



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nitrogen stress treatments. Roots of stressed plants were about 50% larger than roots of control plants at day 6, and accumulation of d.wt was maintained at a sufficient rate that d.wt remained higher than in control plants for 16 days. Although d.wt of roots of nitrogen stressed plants was increased during the initial two weeks of treatment, root morphological development was altered markedly; with increasing nitrogen stress, roots were longer but had much less branching.

If it is assumed that changes in d.wt of plant organs provide an accurate estimate of net carbon flux to the organs, then there was a distinct adjustment in distribution of carbon within nitrogen stressed plants during the first two weeks of treatment (Fig. 4D). Shoot to root d.wt ratios had declined from about 5.0-6.0 in control plants to 3.0 in the 0.1 mM NO_3^- treatment and to 2.0 in the absence of ambient NO_3^- , indicating that a larger proportion of the plant carbon supply was partitioned to the roots with increasing nitrogen stress.

By about 14 d, partitioning of carbon at 0.1 mM NO_3^- was stabilized and the new pattern of distribution was maintained thereafter. In control plants, growth rates of leaves and stems were more rapid than growth rates of roots during the vegetative growth phase (Fig. 4A), so the shoot to root d.wt ratio tended to increase with time (Fig. 4D). This trend was noticeable in plants exposed to 0.1 mM NO_3^- following stabilization of partitioning.

Nitrogen Accumulation and Distribution Within the Plant

Plants exposed to 1.0 mM NO_3^- accumulated nitrogen exponentially throughout the treatment period, while plants exposed to 0.1 mM NO_3^- accumulated nitrogen, but at a slower rate (Fig 5A). The tissue NO_3^- content of stems (Fig 5B) and roots (Fig 5C) continually increased in control plants; however, in nitrogen stressed plants, NO_3^- in stems and roots was rapidly depleted. Tissue NO_3^- in leaves was always very low (less than 0.1 mg N g⁻¹ d.wt of leaves) in all treatment conditions.

With nitrogen stress, there were dissimilar changes in the reduced nitrogen content of the different plant parts. Figure 6A illustrates the increases in reduced nitrogen which occurred in leaves, stems, and roots of control plants. Relative to accumulation of reduced nitrogen in control plants, accumulation in leaves and stems of plants in both nitrogen stress treatments was greatly diminished (Figs 6B and C). Even though accumulation was restricted relative to controls in plants exposed to 0.1 mM NO_3^- , there were real increases in the reduced nitrogen content of leaves and stems throughout the experimental period (data not shown). In contrast to the changes in the shoot, the reduced nitrogen content in roots of nitrogen stressed plants was initially increased. At 0.1 mM NO_3^- , plant roots contained a larger amount of reduced nitrogen than roots of control plants for about 16 d, while in the absence of external NO_3^- , roots had a larger amount for more than 9 days. There is some evidence to suggest that the root content of other mineral nutrients also is initially increased when plants are nitrogen stressed (Ingstad, 1979).

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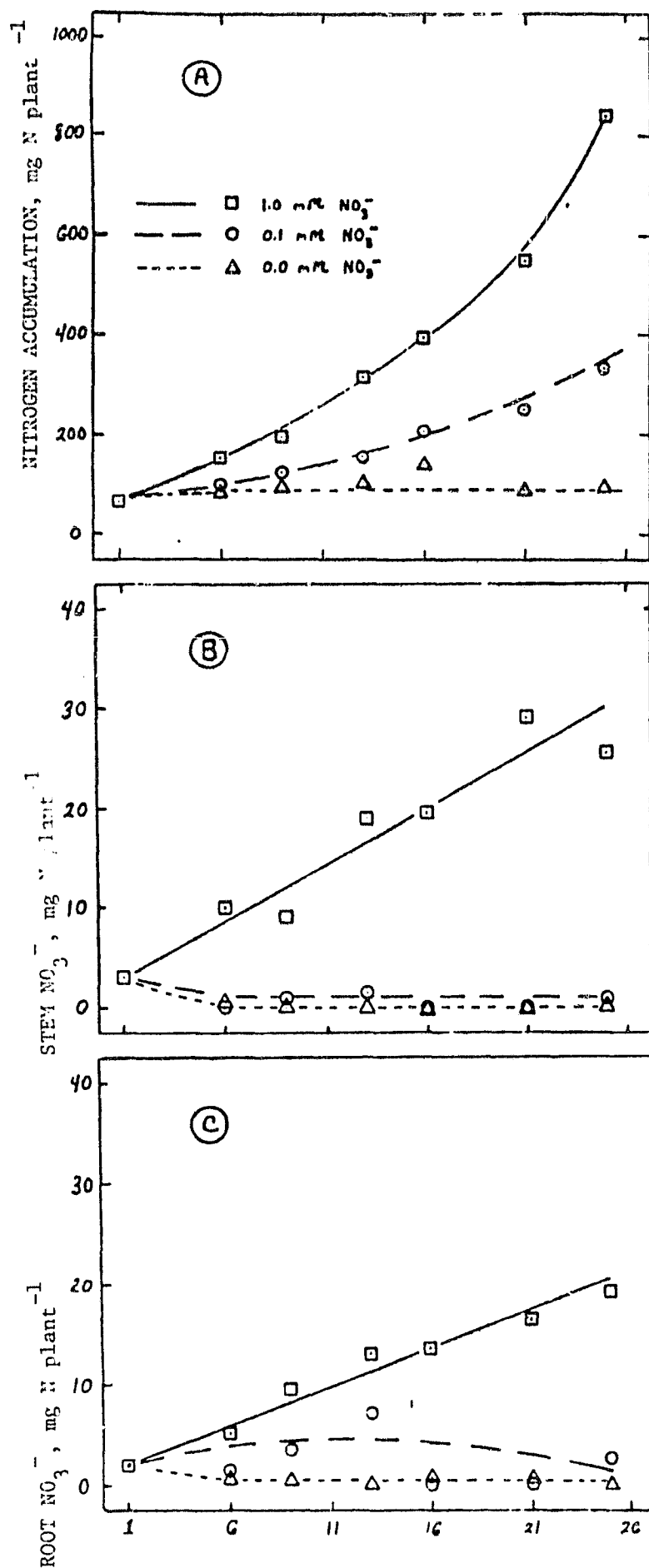
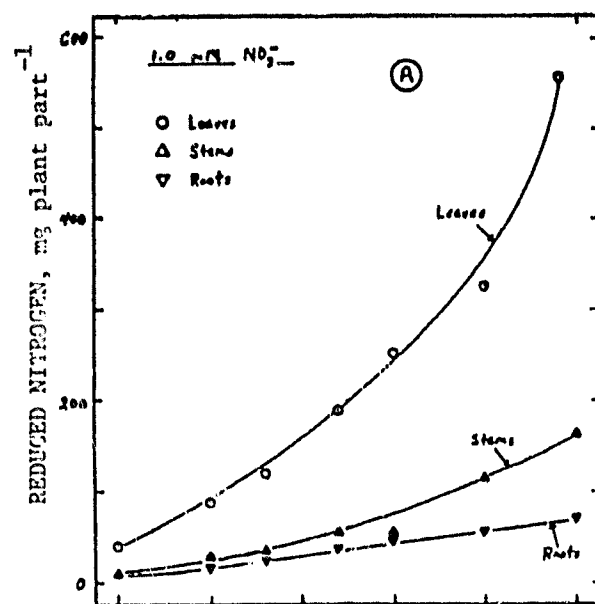


Figure 5



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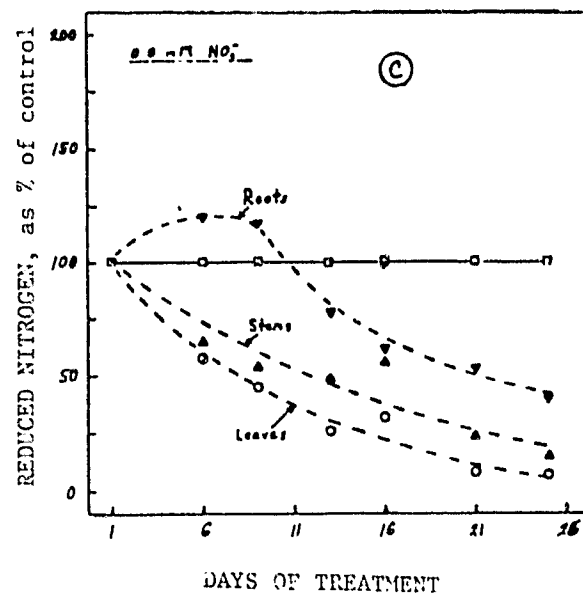
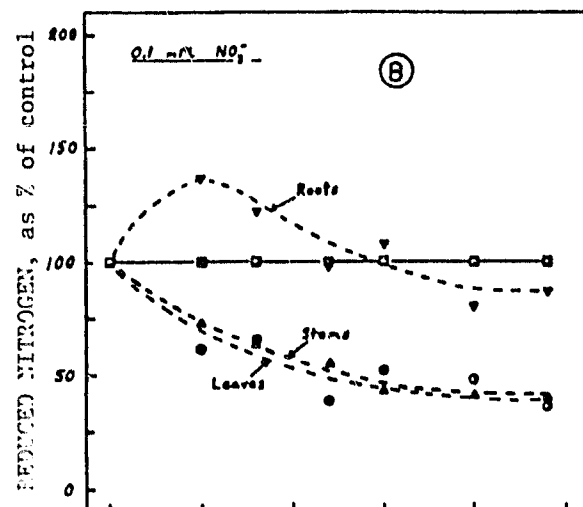


Figure 4

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As was the case with the differential changes in d.wt accumulation, the unequal changes in the reduced nitrogen content of leaves, stems, and roots were associated with definite changes in partitioning of reduced nitrogen within nitrogen stressed plants (Table 1).

In control plants, partitioning of the whole plant supply of reduced nitrogen among plant parts remained relatively stable, with about 69% allocated to leaves, 19% to stems, and 12% to the roots. In plants subjected to nitrogen stress, allocation of reduced nitrogen to leaves was decreased and allocation to roots increased. In the absence of an external NO_3^- supply, plants also tended to increase allocation of plant available reduced nitrogen to stems. Internal NO_3^- pools had been rapidly depleted in these plants, so the changes in proportioning of reduced nitrogen was indicative of considerable remobilization and redistribution out of the leaves. Older leaves, lower in the leaf canopy, became severely chlorotic during the experiment in the absence of external NO_3^- , which is consistent with nitrogen being remobilized predominantly from older leaves (Hill, 1980).

Although the pattern of changes in the reduced nitrogen content of plant organs with nitrogen stress resembled the pattern of changes in dry matter accumulation (compare Figs 6B and C with 4B and C), quantitative differences did exist between the two responses. As a result, the concn of reduced nitrogen in leaves was severely decreased, and the concn in stems and roots also was decreased but to a lesser extent (Table 2).

Table 1. The effects of nitrogen stress on distribution of reduced nitrogen among plant parts. Data are presented as a percentage of the reduced nitrogen content in the whole plant.

Days of Treatment	NO ₃ Treatments									
	1.0 mM			0.1 mM			0.0 mM			Red-N as % of Whole Plant
	Leaves	Stems	Roots	Leaves	Stems	Roots	Leaves	Stems	Roots	
1	70	18	12							-----
6	66	21	13	55	21	24	56	21	23	
9	66	20	14	60	18	22	53	19	28	
13	67	20	13	53	21	25	46	27	27	
16	71	16	13	64	12	24	57	22	20	
21	65	23	11	63	19	18	50	22	27	
25	70	21	9	63	16	21	43	26	31	
Means ± SE	68±2	20±2	12±2	60±5	18±3	22±3	50±6	23±3	26±4	

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Table 2. The effects of nitrogen stress on tissue concentrations of reduced nitrogen over a 25 day treatment period.

Days of Treatments	NO ₃ ⁻ Treatments									
	1.0 mM			0.1 mM			0.0 mM			Red-N as % of Whole Plant
	Leaves	Stems	Roots	Leaves	Stems	Roots	Leaves	Stems	Roots	
	----- Red-N as % of Whole Plant Red-N -----									
1	5.15	1.87	3.12							
6	5.05	1.61	2.45	3.33	1.23	2.18	3.32	1.09	2.03	
9	4.72	1.69	2.22	3.25	0.91	2.05	2.52	0.80	1.86	
13	4.85	1.29	2.48	3.27	1.01	1.97	2.29	0.74	1.18	
16	4.70	0.96	2.41	3.37	0.85	2.04	2.07	0.61	1.24	
21	4.22	0.98	1.90	3.35	0.93	1.84	2.84	0.67	1.32	
25	4.20	0.90	1.34	3.15	0.83	1.77	2.78	0.69	1.28	
Means ±SE	4.70 ±0.37	1.33 ±0.40	2.24 ±0.60	3.29 ±0.08	0.96 ±0.15	1.98 ±0.15	2.64 ±0.44	0.77 ±0.17	1.49 ±0.36	

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DISCUSSION

Alterations in Photosynthesis

Total photosynthetic activity of plants is a function of the rate of CO_2 fixation per unit leaf area and changes in total photosynthetic leaf area per plant. Both of these components were decreased when rapidly growing, vegetative soybean plants were subjected to moderate or acute nitrogen stress.

Restriction of leaf area expansion in nitrogen stressed plants was associated with a multiplicity of growth changes in the shoot. The susceptibility of growth of individual leaves to nitrogen stress, estimated as decreased elongation or expansion, has been well recognized (Watson, 1952; Greenwood, 1976). Less attention has been given to the susceptibility of leaf initiation. Morton and Watson (1948) suggested that the rate of production of new leaves was the most important factor regulating leaf area expansion and net carbon assimilation in plants subjected to nitrogen deficiency. Results from this experiment are consistent with their view, as a similarity was evident among the patterns of leaf emergence (Fig. 3A), leaf area expansion rate (Fig. 3C), and whole plant growth (Fig. 1).

Inhibition of Shoot Growth

The importance of nitrogen availability in the shoot as a determinant of shoot growth and development has been emphasized in schemes developed to explain regulation of whole plant growth (Brouwer and deWit, 1969; Thornley, 1976; Raper et al., 1978). Certainly in this experiment, disruption of growth processes in the leaf canopy was an early growth restriction in nitrogen stressed plants and was a central event in the change in partitioning of carbon within the plant (Fig. 4) as well as in the limitation of expansion of photosynthetic leaf area (Fig. 3) and the

related acquisition of carbon. Since nitrogen stress results in many effects which can influence growth, it is difficult to isolate the particular mechanism responsible for the growth inhibition. It is possible, however, to identify basic factors which are likely to be involved.

The early restrictions of total leaf area expansion and d.wt accumulation in the canopy appeared to be associated with a general decrease in meristematic activity. Emergence (and presumably initiation) of new leaves, lateral stem branching, and growth of individual leaves all declined; therefore, activity at both apical and secondary meristems was affected. Such a response presumably was associated with a decrease in the rate of cell division which would be the expected result of decreased availability of reduced nitrogen. Net increases in genetic material and protein are necessary for cell division, and biosynthesis of these complex nitrogen molecules involves conversion and incorporation of various forms of reduced nitrogen. Consequently, a sustained increase in cell number, i.e. meristematic activity, requires a continuous supply of reduced nitrogen, usually assumed to be translocated to meristematic centers as amino acids (Pate, 1980). It is reasonable that the adjustments in shoot growth in both nitrogen stress treatments were, in general terms, predominantly a reflection of alterations in the rate of supply of amino acids to the meristematic centers.

Growth of individual leaves involves both cell division and cell expansion. Cell expansion is associated with a considerable increase in protein content (Black and Edelman, 1970), and therefore, also would be affected by the rate of supply of reduced nitrogen. In addition, cell expansion could be limited by other factors associated

with nitrogen stress, such as decreased hydraulic conductivity and inability to maintain adequate turgor (Radin and Boyer, 1982).

The Root Response

The early enhancement of growth (Fig 4) and sink strength for nitrogen (Fig 6, Table 1) in roots of nitrogen stressed plants could have originated from an initial restriction of growth in the leaf canopy due to decreased availability of reduced nitrogen, accompanied by increased flux of available carbohydrate to the root. Increased availability of carbohydrate in the root thus could be the primary stimulus of enhanced growth potential in the root and the related "demand" for mineral nutrients. Brouwer (1962) has suggested that in plants subjected to a nitrogen deficiency, absorbed nitrogen is retained in the root and never reaches the shoot. In the 0.1 mM NO_3^- treatment of this experiment, such an explanation is plausible. There is some evidence that increased availability of carbohydrate in the root enhances root reduction of absorbed NO_3^- (Jackson et al., 1980; H \ddot{a} nisch ten Cate and Breteler, 1981). Increased reduction and utilization of nitrogen in the root would account for the observed increase in the proportion of the whole-plant reduced-nitrogen content present in the roots (Table 1).

This explanation, however, cannot account for increased acquisition of reduced nitrogen by roots in the 0.0 mM NO_3^- treatment (Table 1), where no NO_3^- was entering the root system. Endogenous reduced nitrogen was remobilized to root rather than shoot growth sinks. If such redistribution occurs in plants devoid of an external nitrogen supply, then it seems reasonable that it might also occur, perhaps to a lesser extent, when plants are absorbing nitrogen at suboptimal rates. Consistent with this

notion, it has been reported that increased amounts of cotyledonary nitrogen were partitioned to roots of pea seedlings which were acquiring suboptimal amounts of nitrogen from N_2 fixation.

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APPENDIX 3

Nitrogen Assimilation by Soybeans in Response to
Ammonium and Nitrate Nutrition

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A conceptual scheme has been advanced (RAPER et al. 1978) to relate nitrogen uptake activity of roots to a balance between root and shoot functions. Experimental support of this scheme was based on studies in which NO_3^- was the sole external source of nitrogen available for absorption by roots. In many natural and controlled systems, however, a portion of the external nitrogen is supplied as NH_4^+ . Although assimilation of NH_4^+ , which is absorbed readily by plants, may be energetically more efficient than assimilation of NO_3^- (PENNING de VRIES, BRUNSTING, and van LAAR 1974), plants supplied with even moderate concentrations of NH_4^+ alone generally do not grow as well as plants supplied with equal amounts of NO_3^- alone or with NO_3^- and NH_4^+ combined (GIGON and RORISON 1972, HAYNES and GOH 1978).

Suppression of growth by NH_4^+ nutrition can be related in part to the acidification of the root-zone that is associated with the excess influx of cations relative to influx of anions during adsorption of NH_4^+ (KIRKBY and MENGEL 1967). Although acidification of the root-zone can adversely affect root functions and, hence, plant growth (ISLAM, EDWARD, and ASHER 1980; RUFTY, RAPER, and JACKSON 1982), these effects can be avoided with regulation of root-zone acidity by additions of a base or by the presence of a buffer (BARKER, VOLK, and JACKSON 1966).

If root-zone acidity is controlled during uptake of NH_4^+ , the effects of NH_4^+ nutrition on growth can be related to a continued capacity of the plant to supply carbohydrates for amination of absorbed NH_4^+ (NIGHTINGALE 1937; KIRKBY 1968; GIVAN 1979). Certainly, in the absence of a sufficient and continuous supply of carbohydrates for amination of NH_4^+ as it is absorbed, accumulation of nonassimilated NH_4^+ can become toxic to metabolic

processes within tissues (HAYNES and GOH 1978; GIVAN 1979). Based on the scheme proposed for balance between the nitrogen-supplying function of roots and the carbon-supplying function of shoots (RAPER et al. 1978), however, once steady-state growth is attained the uptake of nitrogen is coordinated with the flux of soluble carbohydrate to the roots so that the uptake of nitrogen should not exceed the availability of carbohydrate. An assumption of the scheme is that soluble carbohydrate is rapidly utilized for new growth as it arrives in the root. Since the pool size of carbohydrate in the roots thus remains small (RAPER, WEEKS, and WANN 1976; FARRAR 1981), the energy required for uptake of nitrogen must be utilized concurrently with energy and carbon utilized for growth. If nitrogen is positionally available at the root surface, its uptake thus should be proportional to the continued flux of carbohydrate from the shoot. As nitrogen absorbed by roots is assimilated and translocated to leaves during vegetative growth, it supports initiation and expansion of new leaf photosynthetic area. Addition of this new photosynthetic capacity in turn delimits the flow of carbohydrates to the roots and, consequently, the uptake of nitrogen.

If the balance between carbon and nitrogen supplies remains tightly coupled during growth, there should be no distinction in uptake and utilization of NO_3^- and NH_4^+ . However, as delineated in the scheme the functional balance involves both physiological processes that are reversible on a relatively short time interval and morphological changes that are irreversible and occur over a longer time interval. As a consequence of this separation of processes by time scale, temporary instances can occur when nitrogen is accumulated at rates in excess of

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the current demand by leaf initiation and expansion. If nitrogen is absorbed as NO_3^- , a temporary excess can be stored in any of the several inorganic pools within the plant with minimal impact on other processes. But if NH_4^+ is absorbed even temporarily in excess of current demand by leaf growth, carbohydrate for amination must be diverted from other demands. Although acute NH_4^+ toxicity would be averted by the amination, continued diversions of carbohydrates into stored nitrogenous materials conceivably could limit carbohydrate availability for growth and lead to the slightly increased concentration of assimilated nitrogen sometimes reported for tissues of plants under NH_4^+ nutrition (KIRKBY 1968; HOUBA, van EGMOND, and WITTICH 1971).

The objective of this study was to determine if, when acidity of the root-zone is controlled, the processes involved in balancing root and shoot functions are coupled tightly enough during steady-state growth to prevent suppression of growth with continued absorption of a majority of nitrogen as NH_4^+ . Steady-state growth during vegetative development can be attained over a range of radiation and CO_2 levels in the aerial environment when nitrogen is supplied only as NO_3^- (RAPER et al. 1976, 1978; RUFTY, RAPER, and JACKSON 1981, 1982); however, total dry matter production by plants and partitioning to roots are distinctive for each aerial environment. Thus to determine if the efficiency of NH_4^+ utilization is related to the amount of carbohydrate produced, radiation and CO_2 levels were used to establish different growth potentials.

MATERIALS AND METHODS

Three experiments were conducted with vegetative soybeans [Glycine max (L.) Merrill, 'Ransom'] in the phytotron at North Carolina State University (DOWNS and BONAMINIO 1976). For each experiment, seeds in paper towels were placed in dark germination chambers at 25C and 98% relative humidity and kept moist during germination by capillary action from a 0.1 mM CaSO_4 solution. After three days, 24 seedlings with radicle lengths between 8 and 12 cm were placed into each of three 210-liter continuous-flow, temperature-controlled, hydroponic culture systems with automated control of solution pH (RUFTY et al. 1982). The culture systems were located within a controlled-environment room.

All experiments included a 2-week pretreatment period. The culture solution was maintained at 24 ± 0.5 C and pH was controlled at 5.8 ± 0.1 by additions of 0.01 N H_2SO_4 or 0.01 N $\text{Ca}(\text{OH})_2$. Initial nutrient concentrations in the solution were 1.0 mM NO_3^- , 0.25 mM H_2PO_4^- , 1.25 mM K^+ , 1.0 mM Ca^{2+} , 1.0 mM Mg^{2+} , 2.0 mM SO_4^{2-} , 17.0 μM B, 3.0 μM Mn, 2.2 μM Cl, 0.3 μM Zn, 0.1 μM Cu, 0.04 μM Mo, and 1 mg liter⁻¹ of Fe as Fe-EDTA. The solutions were renewed every two days to avoid depletion effects and concentration of any ion never decreased more than 20%. The controlled-environment room was programmed for day/night temperatures of $26/22 \pm 0.25$ C with a 9-hour day period and a 15-hour night period. During the day period a combination of cool-white fluorescent and incandescent lamps at an input wattage ratio of 10:3 was used to provide photosynthetic photon flux density (PPFD) of $700 \pm 50 \mu\text{E m}^{-2} \text{ sec}^{-1}$.

between wavelengths of 400 and 700 nm and photomorphogenic radiance (PR) of $12 \pm 1 \text{ w m}^{-2}$ between wave lengths of 700 to 850 nm. To effect a long-day floral photoperiod and repress floral expression, the 15-hour night period included a 3-hour interruption after 6 hours by in-ande-scent lamps with PR of 10 w m^{-2} and PPFD of $70 \mu\text{E m}^{-2} \text{ s}^{-1}$. Radiance was measured 95-cm below the lamps with a LI-COR LI-185 Quantum/Radiometer/Photometer^{3/} and LI-1905 and LI-790 sensors. Atmospheric concentration of CO_2 was monitored with a Beckman Model 863 infrared gas analyzer and maintained at $400 \pm 25 \mu\text{l liter}^{-1}$.

Treatments were initiated after the 14-day pretreatment period, when the third trifoliolate leaf had unfolded and the plants had entered the accelerated growth phase described by an exponential function. In each of the three experiments, three sets of plants were exposed to nutrient solutions in which nitrogen was supplied as either 1.0 mM NH_4^+ , 0.5 mM NH_4^+ plus 0.5 mM NO_3^- , or 1.0 mM NO_3^- . The total concentrations of SO_4^{2-} were 2.5, 2.25, and 2.0 mM in the NH_4^+ , NH_4^+ plus NO_3^- , and NO_3^- solutions, respectively. Concentrations of other nutrients were the same as in the pretreatment solution. Solutions were renewed every two days. Temperature of all solutions was 24 C and pH was maintained at 5.8 ± 0.1 .

The aerial environment of the treatment period was different in each experiment. The first experiment was conducted in a continuation of the standard pretreatment environment with PPFD of $700 \pm 50 \mu\text{E m}^{-2} \text{ sec}^{-1}$ and CO_2 level of $400 \mu\text{l liter}^{-1}$; the second experiment was conducted in a low radiance environment with PPFD of $325 \pm 25 \mu\text{E m}^{-2} \text{ sec}^{-1}$

^{3/} Trade names are given as a part of the exact experiment conditions and not as an endorsement to the exclusion of other products.

and CO_2 level of $400 \mu\text{l liter}^{-1}$; and the third experiment was conducted in a high CO_2 environment with PPFD of $700 \mu\text{E m}^{-2} \text{sec}^{-1}$ and CO_2 level of $1000 \mu\text{l liter}^{-1}$. Photoperiods, including the 3-hour interruption of the night period, and day/night temperatures were the same as in the pretreatment.

In all experiments, six groups of six randomly selected plants were sampled at the initiation of treatments. During the treatment periods of 27, 28, and 26 days in standard, low radiance, and high CO_2 environments, three or four plants were sampled from each treatment solution at intervals of four to six days. Individual plants were separated into leaves, stems (including petioles), and roots. Leaf areas were measured photometrically. All tissues were frozen, freeze-dried, weighed, and ground for analysis.

Total nitrogen was assayed by a Kjeldahl procedure modified to digest all nitrogenous materials, including NO_3^- , to NH_4^+ (NELSON and SOMERS 1973) for colorimetric determination (CATALDO, SCHRADER, and YOUNGS 1974). Hot water extracts of tissues were analyzed for NO_3^- nitrogen by a manual modification of the method of LOWE and HAMILTON (1967). Reduced nitrogen was calculated as the difference between total and NO_3^- nitrogen. The NH_4^+ in tissues was extracted with redistilled water for 12 hours. In a procedure adapted from CONWAY (1957), an aliquot of the extract was placed into a 25 mM Na-tetraborate buffer at pH 10 and 55 C and NH_3 diffused for 48 hours into 3.0 N H_2SO_4 . The NH_4^+ then was determined colorimetrically as before.

Increases in dry matter, nitrogen, and leaf area during the treatment periods were described by the regression equation $\ln W = a + b(t)$ where W is weight of dry matter for whole plants or plant parts, of nitrogen, or area of leaves and t is days after initiation of treatments.

The regression coefficient b for the respective measured parameters is equivalent to relative growth rate (RGR) for whole plants and plant parts and relative accumulation rate of nitrogen (RARN) (RAPER et al. 1978). The correlation coefficient r for all fitted regressions exceeded 0.985.

RESULTS AND DISCUSSION

In the culture conditions used in these experiments, newly germinated soybean seedlings remain in a slower "lag" phase of growth for 7 to 10 days. Seedlings were grown in a nutrient solution with NO_3^- as the nitrogen source for a 14-day pretreatment period and, thus, were into an accelerated, steady-state growth phase when first exposed to treatment solutions containing NH_4^+ only or NH_4^+ combined with NO_3^- . As in previous experiments (RUFTY et al. 1981), for soybeans continuously exposed to NO_3^- as a sole nitrogen source relative growth rates of plants (table 1) were lower in the low PPFD treatments ($325 \mu\text{E}\cdot\text{m}^{-2}\text{s}^{-1}$ PPFD and $400 \mu\text{l}\cdot\text{l}^{-1}$ CO_2) and higher in the high CO_2 treatment ($700 \mu\text{E}\cdot\text{m}^{-2}\text{s}^{-1}$ PPFD and $1000 \mu\text{l}\cdot\text{l}^{-1}$ CO_2) than in the standard environment ($700 \mu\text{E}\cdot\text{m}^{-2}\text{s}^{-1}$ PPFD and $400 \mu\text{l}\cdot\text{l}^{-1}$ CO_2). These changes in growth rate, however, did not change the ability of the plants to utilize NH_4^+ relative to NO_3^- . Within each aerial environment, whole plant growth in the presence of NH_4^+ alone or NH_4^+ combined with NO_3^- continued at rates nearly identical to plants grown with NO_3^- as the nitrogen source (fig. 1, table 1). Also, though the rates of emergence and expansion of leaves (data not shown) and dry matter accumulation of leaves (fig. 2) and roots (fig. 3) varied as expected among aerial environments (RUFTY et al. 1981), there were no distinctions among plants within an aerial environment attributable to the presence of NH_4^+ as all or part of the nitrogen source.

The efficiency of NH_4^+ utilization was not related simply to the rate of photosynthate movement to the root systems. Assuming root dry weight to be proportional to the amount of carbohydrate transported to the roots, by the end of the treatment period cumulative transport for plants grown at low PPFD was half of that for plants grown in the

Table 1. Relative growth rates (RGR) and intercepts (a) fitted by regression analysis to dry weights of soybean plants grown in different aerial environments and with different combinations of NH_4^+ and NO_3^- in a complete nutrient solution. The regression model was $\ln \text{Dry Weight} = a + b (\text{day})$ where b is the RGR.

AERIAL ENVIRONMENT	NITROGEN SOURCE		RGR	a
	NO_3^-	NH_4^+		
	----- mM -----		$\text{g} \cdot \text{g}^{-1} \cdot \text{day}$	g
Standard	1.0	0.0	0.120	0.031
	0.5	0.5	0.124	-0.030
	0.0	1.0	0.121	0.049
Low PPFD	1.0	0.0	0.110	-0.320
	0.5	0.5	0.109	-0.364
	0.0	1.0	0.115	-0.327
High CO_2	1.0	0.0	0.142	-0.328
	0.5	0.5	0.141	-0.149
	0.0	1.0	0.141	-0.194

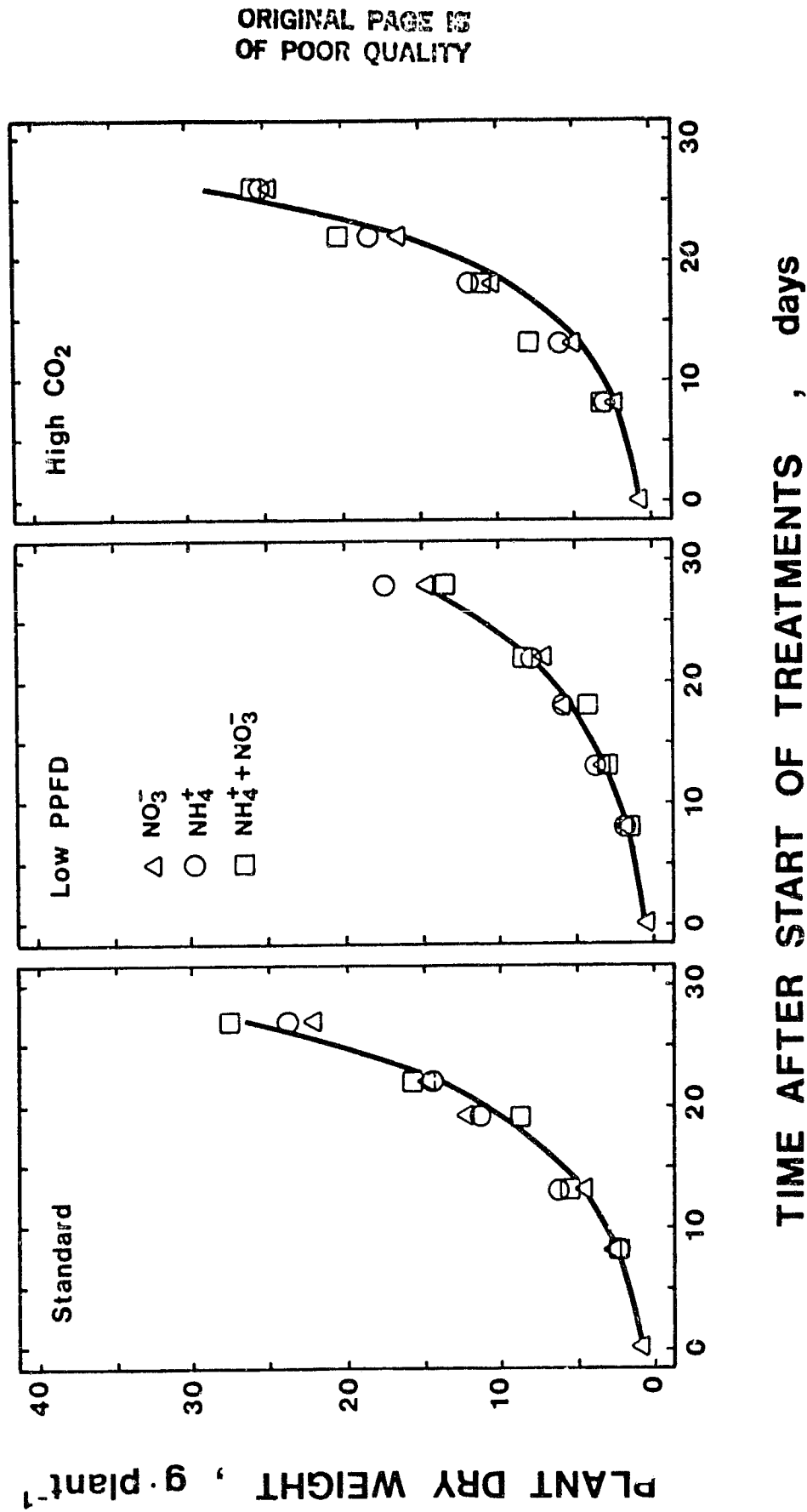


FIGURE 1

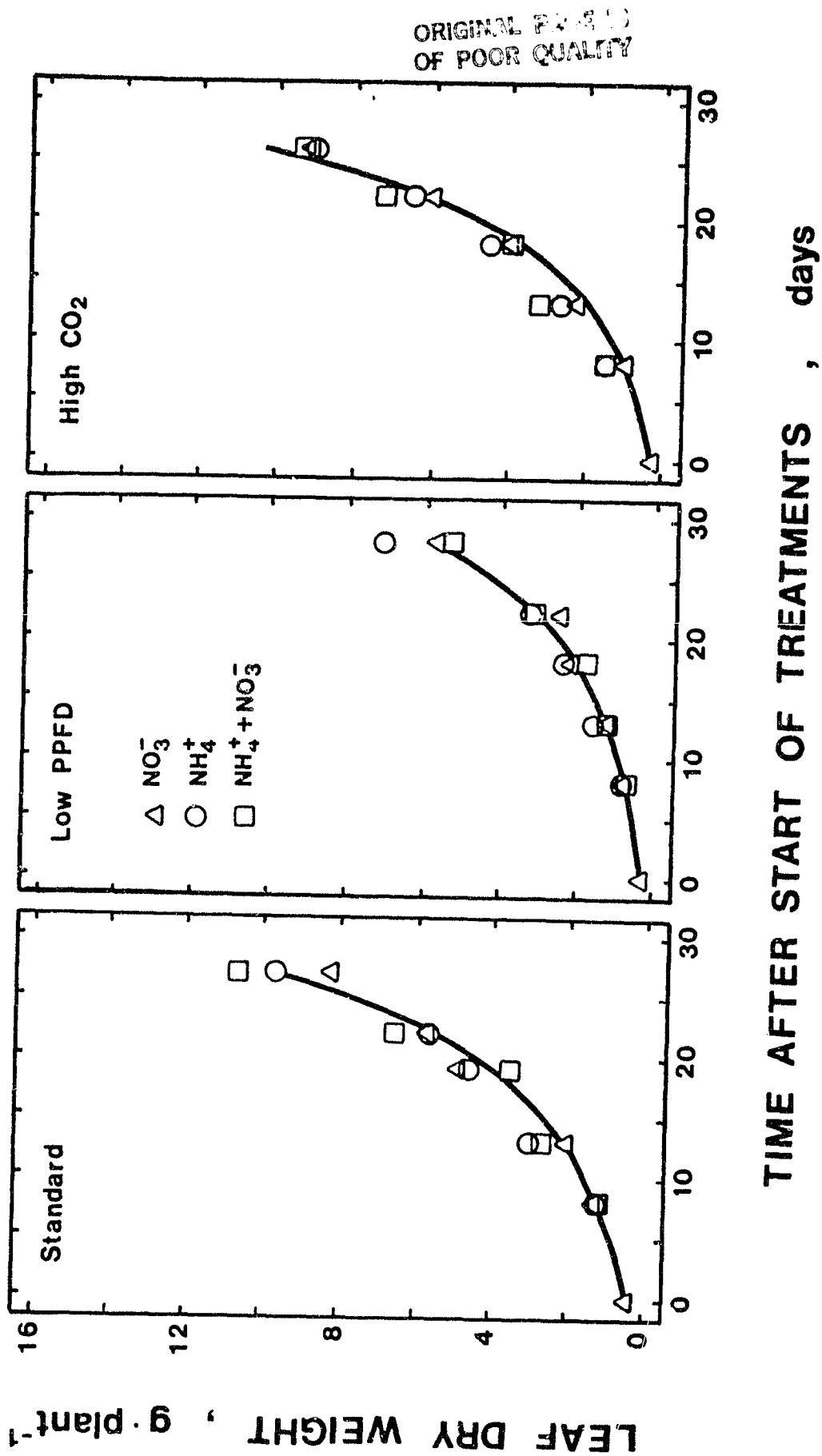
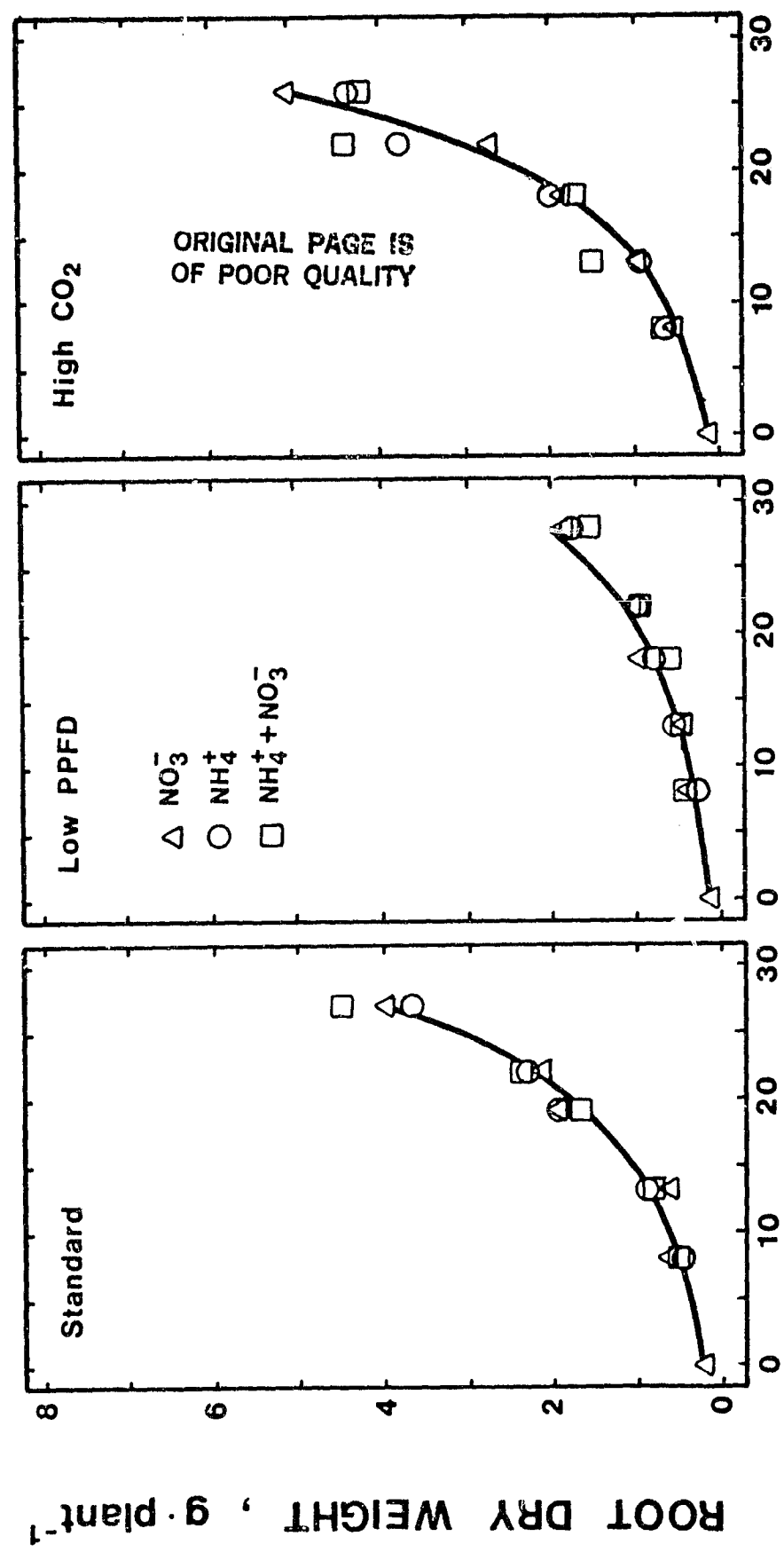


FIGURE 2



TIME AFTER START OF TREATMENTS, days

FIGURE 3

standard environment and a third of that for plants grown under CO₂ enrichment (fig. 3). Also, the proportion of total plant dry weight partitioned to roots was lowest for plants grown at low PPFD with shoot to root ratios at the end of the treatment period of 7.9 ± 0.9 , 5.7 ± 0.2 , and 4.9 ± 0.1 for low PPFD, standard, and high CO₂ environments, respectively. Nevertheless, even for low PPFD, NH₄⁺ as a sole nitrogen source did not suppress plant growth relative to NO₃⁻.

Under steady-state conditions the uptake of nitrogen as NH₄⁺ is as effectively coordinated with flux of soluble carbohydrate to the roots as the uptake of NO₃⁻. Nitrogen accumulation in plants differed among the three aerial environments (fig. 4), but within each aerial environment plants supplied with NH₄⁺ as the sole nitrogen source accumulated nitrogen at rates similar to those grown in NO₃⁻ alone or in combination of NH₄⁺ and NO₃⁻. As shown by LEWIS et al. (1982), NH₄⁺ apparently was aminated rapidly in the roots as it was absorbed since concentrations of NH₄⁺ ion in tissues from all nitrogen treatments were less than $0.7 \mu\text{g NH}_4^+ \cdot (\text{g dry weight})^{-1}$ in the roots and other plant parts. Thus, even for the low rates of dry matter productivity under the low PPFD aerial environment (fig. 1), sufficient carbohydrate was available for continued amination of absorbed NH₄⁺.

In tissues of plants exposed only to NH₄⁺ during the treatment period, the amount of NO₃⁻ rapidly declined (fig. 5) while NO₃⁻ continued to be accumulated in plants exposed to NO₃⁻-containing solutions. Although a portion of the NO₃⁻ in roots of the NH₄⁺-fed plants could have been effluxed, the decline of NO₃⁻ content also occurred in leaf and stem tissues. This indicates that NO₃⁻ previously accumulated during the pretreatment period remained in a pool available, albeit slowly, for reduction.

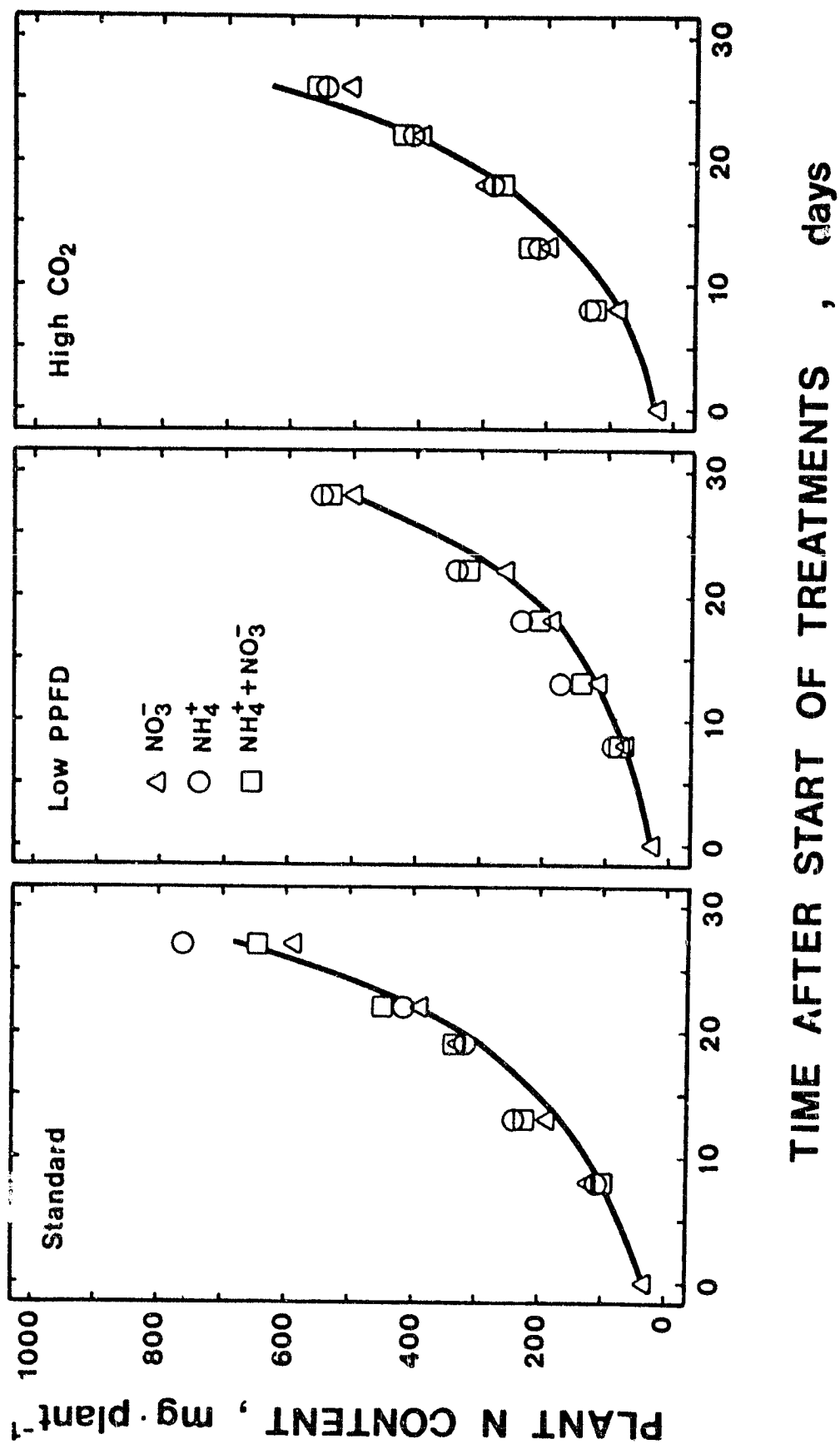


FIGURE 4

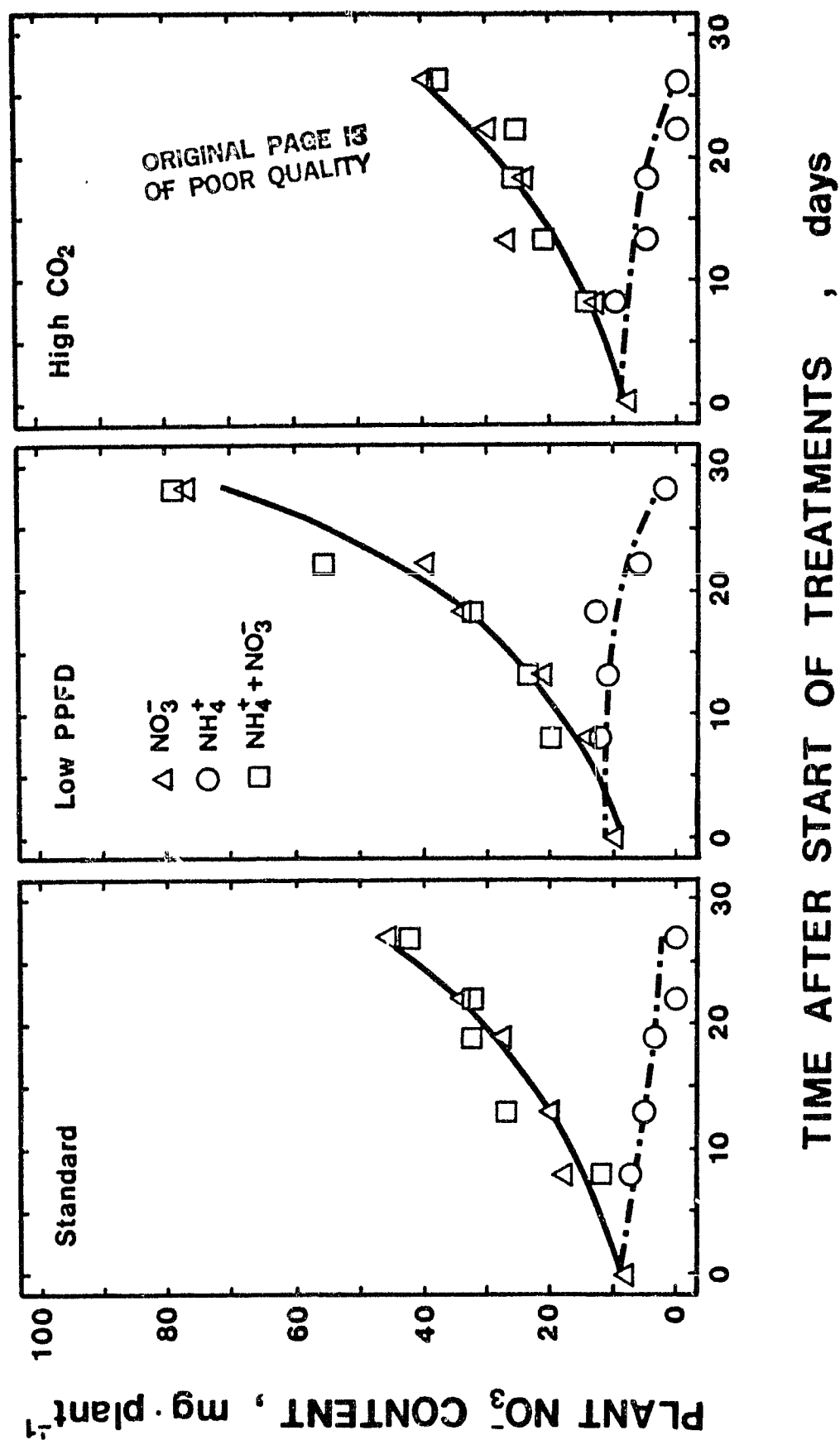


FIGURE 5

CONCLUSIONS

Neither toxicity nor suppression of growth occurred for NH_4^+ -fed plants at the distinctive steady-state growth rates of this study, although the concentration of NH_4^+ used in the nutrient solutions was similar to concentrations reported in other studies to suppress the growth of soybeans (PARK and STUTTE 1973; LEHAV, HARPER, and HAGEMAN 1976) and other species (KIRKBY and MENGEL 1967; GIGON and RORISON 1972; COX and REISENAUER 1973; McELHANNON and MILLS 1978). These results support the contention that presence of NH_4^+ as a nitrogen source does not alter the interdependency of root and shoot functions in determining nitrogen uptake and utilization during steady-state growth. Since the physiological and morphological processes involved in balancing root and shoot functions apparently are coupled tightly enough under steady-state conditions to prevent suppression of growth with continued adsorption of a majority of nitrogen as NH_4^+ , adverse effects of NH_4^+ supply on growth must involve other factors that were avoided in this study.

Environmental conditions for this study were rigidly controlled during the experiments. Root-zone acidity was constantly monitored and controlled at the same pH among treatments to avoid possible effects on root growth and function. Although radiation and CO_2 regimes were imposed to alter growth responses, these environmental regimes were stable and constantly maintained during the treatment period so that plants were able to attain steady-state growth under each regime. Since under these carefully defined and controlled environments differences in growth did not occur for NH_4^+ and NO_3^- nutrition, we

postulate stresses, such as plant water deficit, which alter availability of carbohydrates for transport to roots separately from the uptake and utilization of nitrogen can contribute to instances of growth suppression under NH_4^+ nutrition. By limiting photosynthetic activity and leaf expansion independently of nitrogen availability, such stresses could limit the continued availability of carbohydrates for amination of absorbed NH_4^+ .

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APPENDIX 4

A Dynamic Model for Plant Growth:
Response to Changing Temperatures

Mien Wann

C. David Raper, Jr.

INTRODUCTION

Temperature is among the most pervasive environmental factors affecting plant growth. If a model of the influence of temperature on plant growth is to be useful, the direct effects of temperature on essential physiological processes must be identified and precisely determined. In a dynamic simulation model being developed for plant growth (Wann, Raper, and Lucas, 1978; Wann and Raper, 1979), we have incorporated the effects of ambient temperature through the processes of photosynthesis, respiration, growth, and aging. We have assumed, however, that translocation processes for carbohydrates between source and sink are not directly dependent on temperature (Wardlaw, 1974), but rather that translocation rates are responsive to temperature indirectly through the effects of temperature on the concentration of carbohydrate in the source pool and the size and metabolic activity of the receiving (sink) organ.

The dry matter accumulation and distribution predicted by the model corresponded closely to the measured values of plants grown under a wide range of constant temperatures in controlled-environment rooms. A temporal change of ambient temperatures, however, can alter dry matter accumulation (Fitter and Hay, 1981). Before the model can be useful for either research or practical applications, it must have a demonstrated capability to predict behavior of plants under conditions of changing temperatures. In the present paper, we present experimental results of the capacity of the model to predict growth of tobacco plants when controlled temperatures were changed at weekly intervals in an otherwise constant environment.

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MATERIALS AND METHODS

Two experiments were conducted in the phytotron at North Carolina State University (Downs and Bonaminio, 1976) to measure dry matter accumulation and distribution in a flue-cured cultivar of tobacco (Nicotiana tabacum L., cv. NC 2326) in response to temporal changes in ambient temperature. In the first experiment, temperatures were changed at weekly intervals, and in the second experiment, temperatures were changed at daily intervals. Environmental conditions other than temperature were common to both experiments.

Environmental and cultural conditions

Tobacco seedlings, grown as described previously (Raper, Weeks, and Wann, 1976), were transplanted into individual 25.4-cm diameter, 9 liter plastic pots filled with sand when four leaves with areas greater than 17 cm^2 had developed. Plants were watered daily during the first and fifth hour of the day period with 600 ml of deionized water. During the final hour of the day period, 1200 ml of deionized water was applied to each plant, and after the excess water had drained, 600 ml of a complete nutrient solution (Raper et al., 1976) was applied. Neither additional water nor nutrient was applied during the night period.

During the 9-hour day period, a combination of cool white fluorescent and incandescent lamps at an input wattage ratio of 10:3 provided photosynthetic photon flux density (PPFD) of $735 \pm 25 \mu\text{mol m}^{-2}\text{s}^{-1}$ between wavelengths of 400 and 700 nm and photomorphogenic radiation (PR) of $12 \pm 1 \text{ W m}^{-2}$ between wavelengths of 700 and 850 nm. The 15-hour night period included a 3-hour interruption between the sixth and ninth hours by the incandescent lamps alone to establish long-day floral photoperiods (Thomas et al., 1975). The incandescent lamps provided PR of 11 W m^{-2} and

PPFD of $69 \mu\text{mol m}^{-2}\text{s}^{-1}$. Radiation was measured 95 cm below the lamps with a cosine corrected LI-COR^{1/} quantum/radiometer/photometer and sensors. Atmospheric concentrations of carbon dioxide was monitored by infrared gas analysis and maintained between 375 and 400 $\mu\text{l liter}^{-1}$ by injection of commercial grade carbon dioxide.

Ambient temperatures during the experiments were monitored constantly with a welded-bead thermocouple in a shielded, aspirated housing and were maintained within 0.25 C of the set point. Transitions in temperature between day and night periods were abrupt and coincident with transition of radiation.

Weekly changes in temperature

Separate growth rooms were programmed at day/night temperatures of 18/14, 22/18, 26/22, 30/26, and 34/30 C. Sets of 45 plants were placed under each of these initial temperatures and at weekly intervals for five weeks were rotated progressively through the temperatures in a descending sequence. Subsequent sets of 45 plants placed under each of the initial temperatures were rotated at weekly intervals progressively through the temperatures in an ascending sequence (see inserts of Figs. 1 to 5). When plants in the descending sequence reached the lowest temperature, 18/14 C, they were rotated to the highest temperature, 34/30 C, and when plants in the ascending sequence reached the highest temperature, they were rotated to the lowest temperature. Beginning on the day of transplanting into the initial temperatures, three randomly selected plants were sampled from each group at the beginning of the day period of every Monday, Wednesday, and Friday during the experiment. Sampled plants were separated into leaf, stem, and root components, leaf area calculated from linear measurements

(Raper, Smith, and York, 1974), and the components frozen, freeze-dried, and weighed. The data for individual plants sampled on the same day were kept separate for estimation of standard deviations.

Daily changes in temperature

A set of 52 plants was placed at transplanting into a single chamber. For the first 13 days of growth, the chamber was programmed at 34/30 C. Beginning on Day 14, day and night temperatures were changed daily by 4 C according to the schedule of 34/30 C on Day 14, 30/26 C on Day 15, 26/22 C on Day 16, 22/18 C on Day 17, and 18/14 C on Day 18. On Day 19, the sequence was reversed so that beginning with 18/14 C on Day 19 temperature was increased to 34/30 C on Day 23. The descending and ascending cycle was repeated between Days 24 and 33 and then kept at 34/30 C for Days 34 and 35 (see inset of Fig. 6). All temperature changes were coincident with the light and dark transitions. Starting on Day 14, four randomly selected plants were sampled at the beginning of the light period of days with temperatures of 34/30, 26/22, and 18/14 C. Plants were sampled and measured the same of those in the first experiment.

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RESULTS AND DISCUSSION

To test the predictive capacity of a dynamic model being developed for plant growth (Wann et al., 1978; Wann and Raper, 1979), we carried out simulations of the dry matter accumulation in the plant parts, as well as the whole plant, of tobacco using the programmed changes in temperature and the initial plant conditions measured in the phytotron experiments. All other parameter values used for the simulations were adopted directly from the previous description of the model for tobacco (Wann et al., 1978) without adjustment. Since environmental conditions other than temperature did not vary among treatments, the behavior of the model can be compared to the actual growth of plants as a function only of temperature.

The behavior of the model generally was similar to the measured growth of whole plants for both descending and ascending temperature sequences at weekly (Figs. 1 to 5) and daily (Fig. 6) frequencies of change. This indicates that, when other environmental conditions remain constant, the model is capable of reasonably predicting the total dry matter production under changing temperatures with a switching interval as short as one day.

The response of a plant to changing temperatures can be considered as two consecutive phases. In the initial phase, there may be a delay between the time of change in temperature and the time when growth rate of the plant begins to react. In the second phase, there may be a time response for the growth rate itself. In carrying out the simulations for this study, we assumed that the change in growth rate was instantaneous with changes in temperature. Since there was close overall agreement between these simulations and the actual growth measured under the

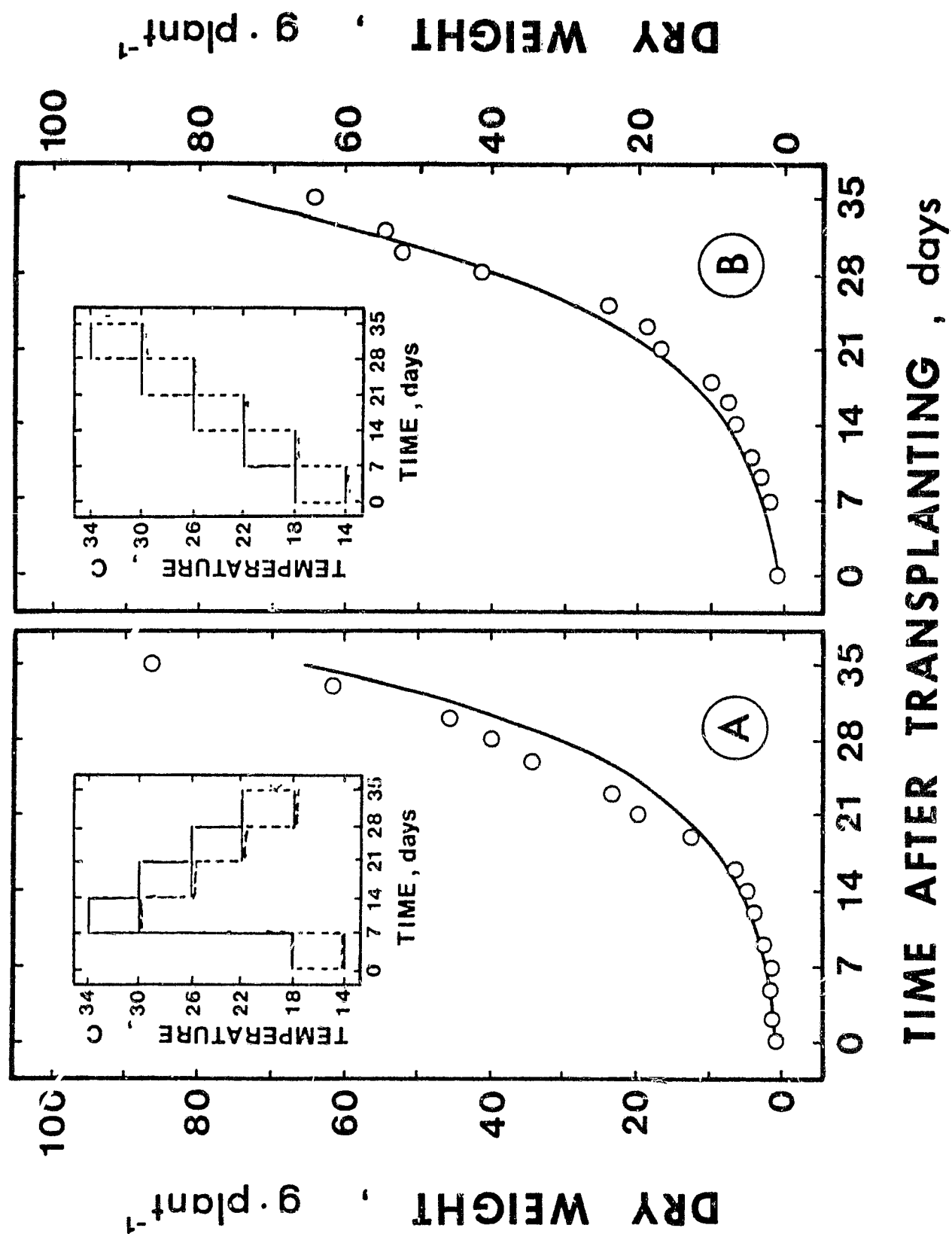


FIGURE 1

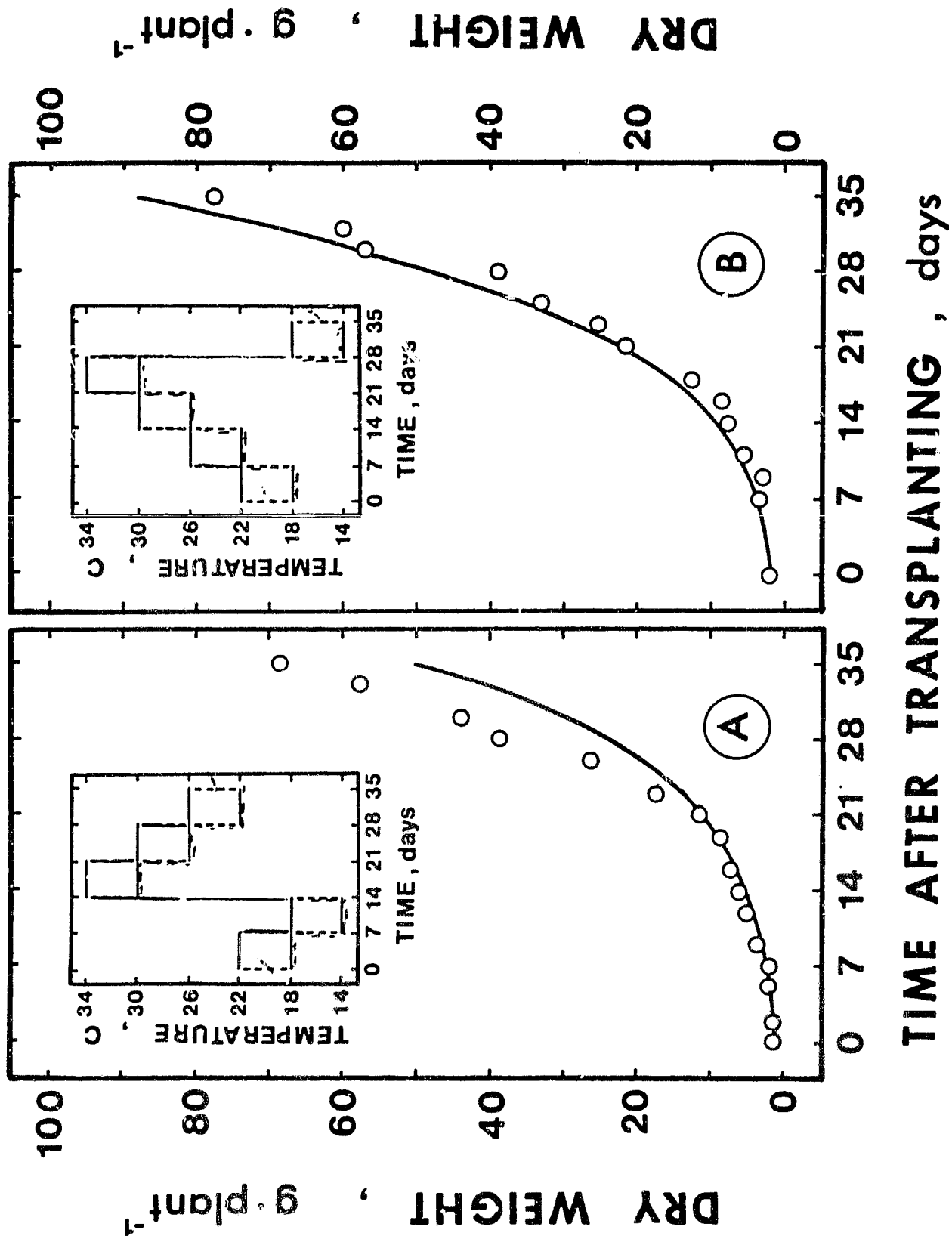


FIGURE 2

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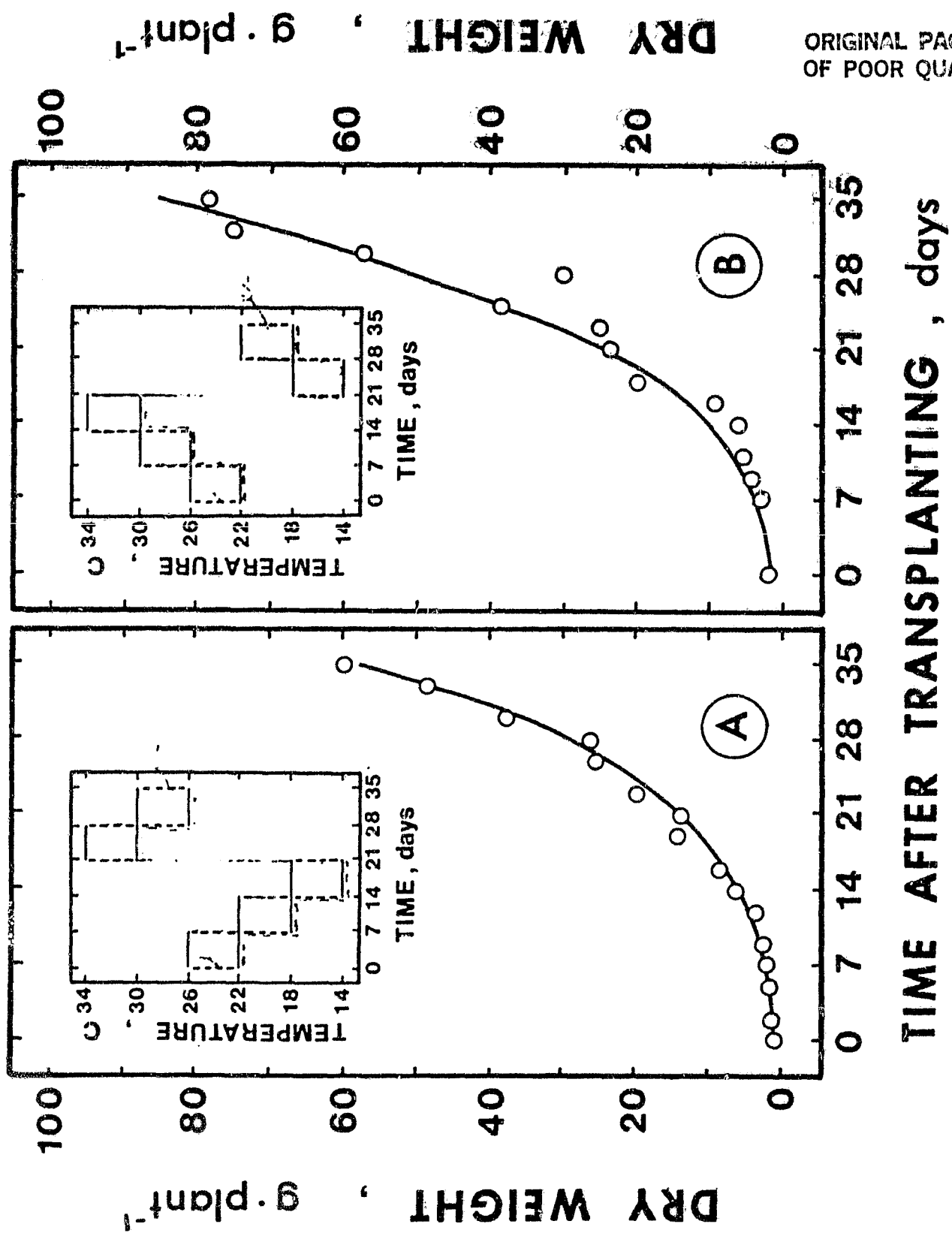


FIGURE 3

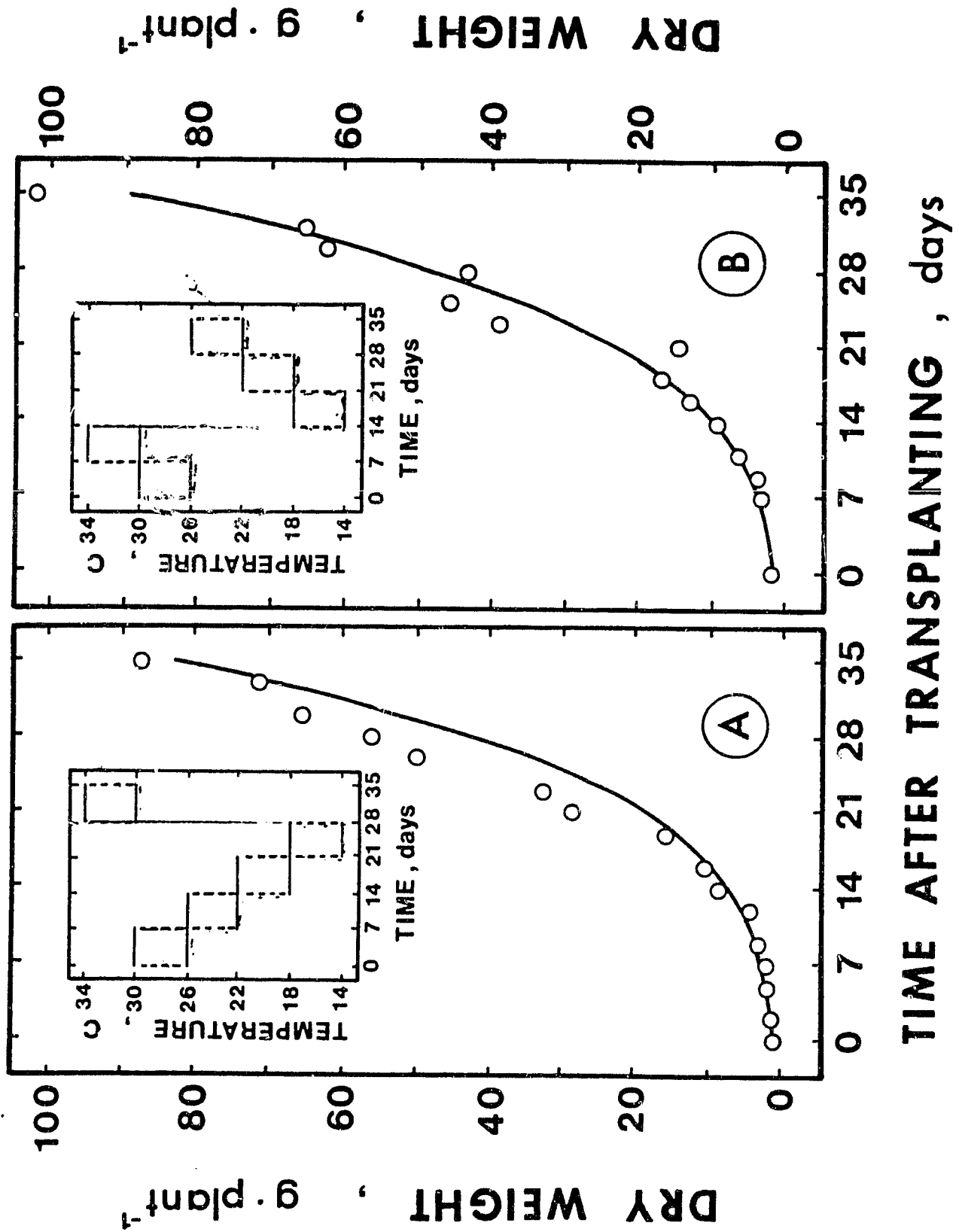


FIGURE 4

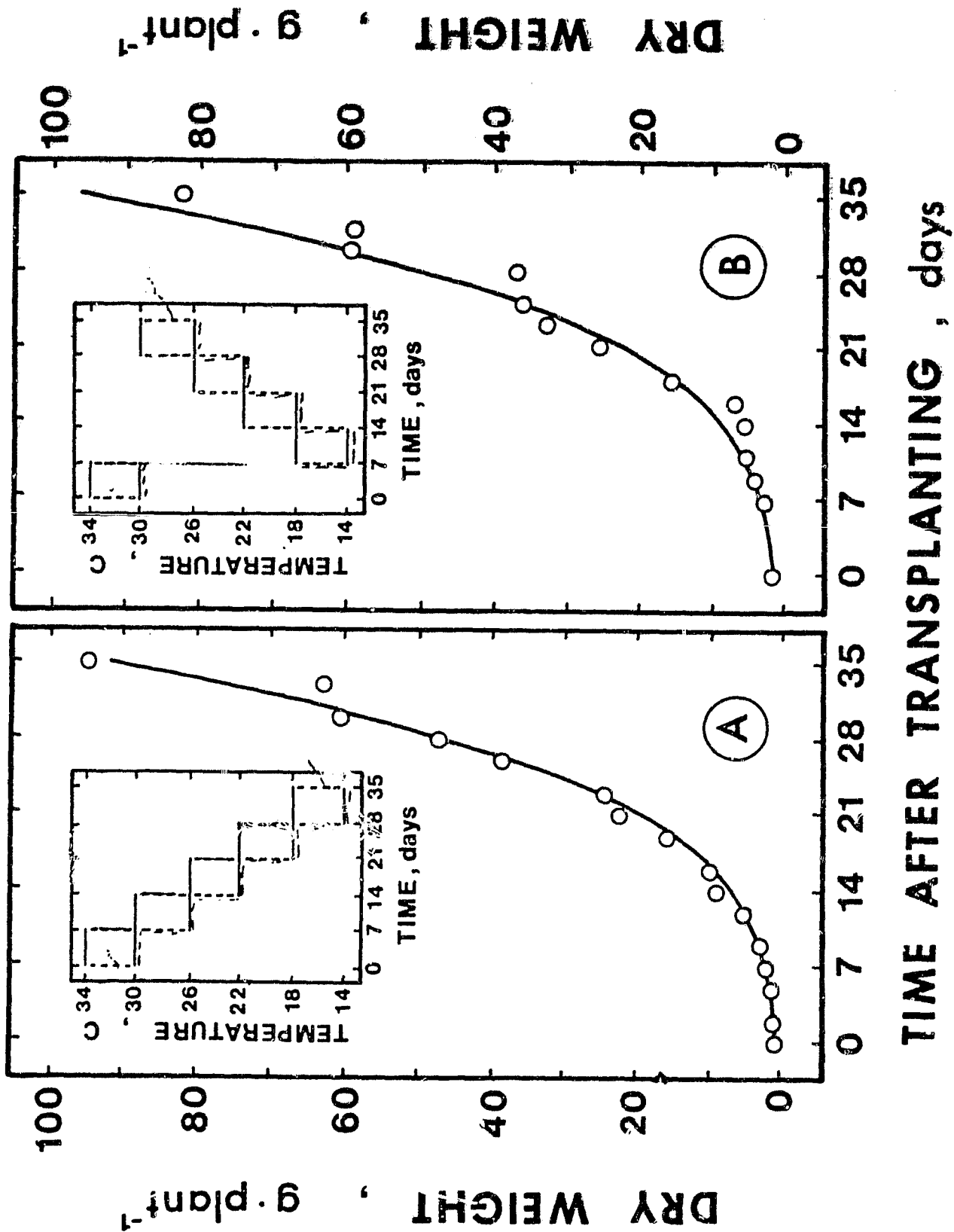


FIGURE 5

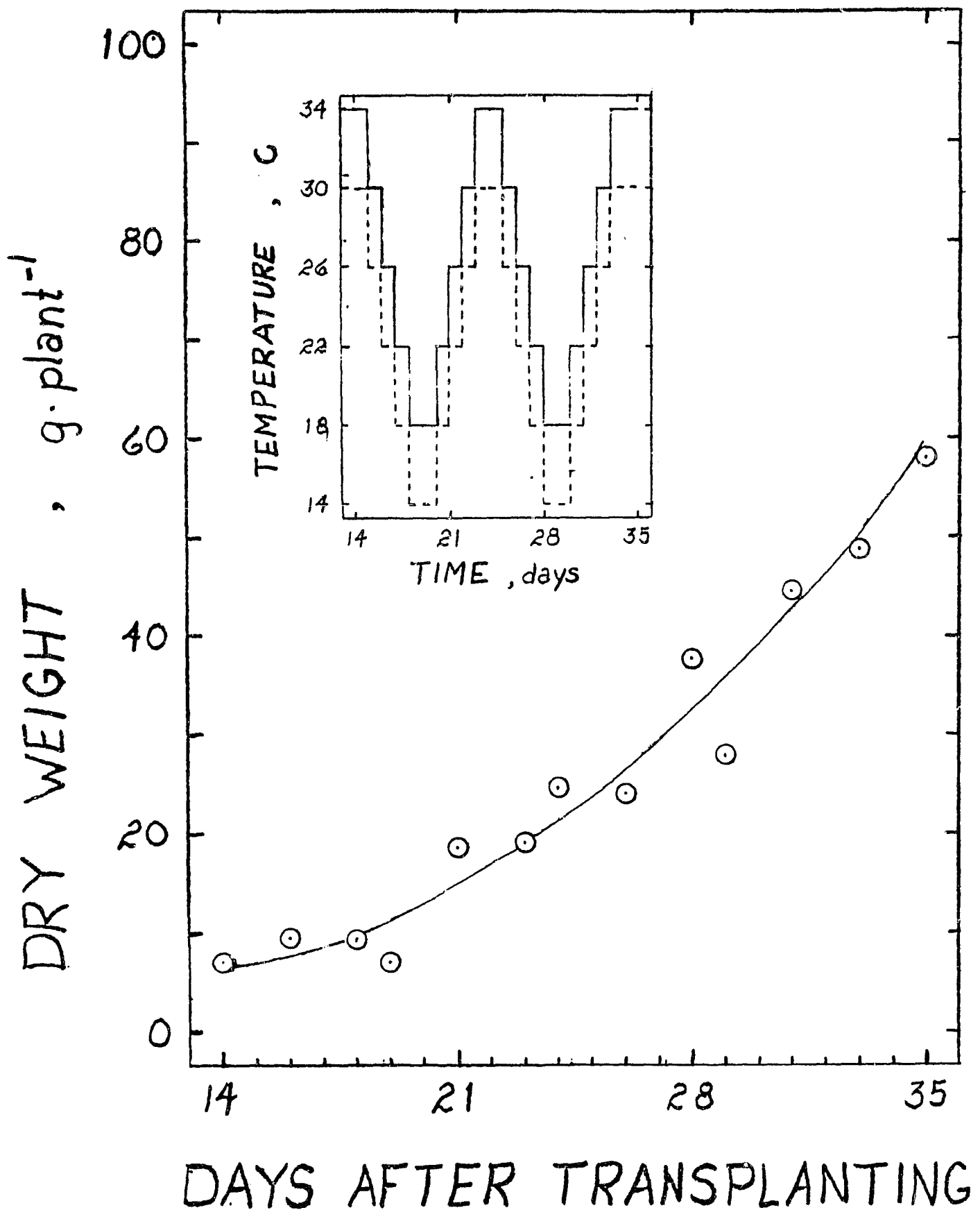


FIGURE 6

temperature programs, we conclude that the delay in time for response plus the change in response time of growth combined are shorter than the one-day time resolution observable in the study.

A second assumption that we accepted in carrying out the simulations was that changes in aerial and root-zone temperatures were synchronous and equal. If the changes in aerial and root-zone temperatures are not synchronous, the responses in growth rates of shoots and roots should be out of step, and since distribution of dry matter between shoot and roots would be affected by the respective growth rates of these organs, the shoot to root ratios should be modified. The extent of the modification in shoot to root ratios would depend upon the duration of the lag between changes in aerial and root temperatures, the sequence of changes in temperature, and the frequency of changes. Measurements of root-zone temperature in other phytotron experiments under similar conditions (unpublished data) indicate that a delay between changes in aerial and root temperature of one to two hours can occur. When temperatures were changed at weekly intervals, the effects of a time lag of less than two hours between aerial and root temperatures would not be observable considering the coefficient of variation of about 10% in measured dry matter. Even when temperature was changed at daily intervals, only a slight difference was observable between predicted and measured shoot to root ratios. The assumption of synchronous changes in shoot and root temperatures appears to be acceptable for these experimental conditions.

Based on the results of this study, we conclude that the assumption of temperature independence of translocation processes is adequate for the purpose of modeling plant growth over the experimental range of temperatures of 34 to 14 C. We also conclude that plant growth in controlled environ-

ments approximately tracts variations in temperature with frequencies of less than 0.5 day. For higher frequencies of temperature change, such as normally occur in field environments, additional investigation is necessary before the model can be applied with assurance.

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